IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	William P. Spencer, et al.
Appl. No.	:	10/805,386
Filed	:	March 22, 2004
Title	:	ESTERIFIED FATTY ACID COMPOSITION
Examiner	:	D. Carr
Group Art Unit	:	1621
Conf. No.	:	1731

DECLARATION OF THOMAS VAN DYKE
SUBMITTED UNDER 37 C.F.R. § 1.132

I, Thomas Van Dyke, DDS, declare as follows:

1. I am a professor at the Department of Periodontology and Oral Biology, Boston University Goldman School of Dental Medicine. I also serve as a scientific advisor for Imagenetix, Inc., the assignee of the above-captioned patent application.

2. I received my D.D.S. (1973) from Case Western Reserve University; M.S. (1979) from State University of New York (SUNY) at Buffalo in Oral Sciences and my Ph.D. (1982) from SUNY at Buffalo in Oral Biology. I received the International Association for Dental Research (IADR) Award for Basic Research in Periodontology in 2001 and the Norton Ross Award for Excellence in Clinical Research in 2002. I serve or have served on the editorial boards of Infection and Immunity (1989-present); Journal of Periodontology (1988-present), Journal of Periodontal Research (1987-present); Journal of Clinical Periodontology (1991-1995), Journal of Public Health Dentistry (1991-1995), Current Opinions in Periodontology (1993). I served as President of the Periodontal Diseases Research Group of the IADR from 1991-1992. I have authored/co- authored over 200 original articles, and numerous abstracts and book chapters. I am a member of the American/International Association of Dental Research, American Academy of Periodontology, American Dental Association, and a Diplomate of the American Board of Periodontology. My research interests are the structural and functional relationship of abnormalities of the inflammatory process with focus on regulation of phagocytic cells, in the etiology and pathogenesis of periodontal diseases. I am well-known in the scientific community for my work on the pathways of resolution of inflammation and pathogenesis of periodontal diseases, neutrophil biology, and clinical research.

3. I have read and understood the specification of the above-captioned patent application, the currently pending claims as submitted to the U.S. Patent and Trademark Office (PTO) on August 9, 2007, and the most recent Office Action, mailed by the PTO on October 22,

2007. I have also read and understood a declaration written and signed by Dr. Robert Hesslink on March 21, 2006, and filed with the PTO on March 23, 2006 (the Hesslink Declaration).

4. The mouths of humans and other mammals comprise a large number of different bacteria, which, among other functions, aid with the digestion of the food as it is chewed and mixed with saliva. The presence of normal populations of these bacteria do not cause any harm to the individual.

5. However, if certain strains of the normally present bacteria grow to abnormally high populations in certain areas of the mouth, for example at the gum line, the bacteria cause inflammatory conditions in the periodontium, i.e., the tissues that support the teeth in the mouth. The inflammation of the periodontium, also known as gingivitis, can lead to periodontal disease, or periodontitis, which includes the progression from gingivitis to erosion of tooth bone, and ultimately to tooth loss. Periodontal disease, therefore, is an inflammatory disease.

6. It has been known for some time that the administration of anti-inflammatory drugs can aid in the treatment of periodontal disease. For example, the use of flurbiprofen (trade name ANSAID®), a non-steroidal anti-inflammatory drug primarily used for the treatment of arthritis, has been studied for its efficacy in the treatment of periodontal disease. See, for example, Heasman et al., J. Clin. Periodontol 1993; 20:457-464, "The use of topical flurbiprofen as an adjunct to non-surgical management of periodontal disease," attached hereto as *Exhibit A*.

7. However, the use of anti-inflammatory drugs is fraught with the onset of adverse side effects. Gastrointestinal ulcers and bleeding, bleeding at other tissues, and cardiac episodes are among some of the adverse events observed with troubling frequency during the use of anti-inflammatory medications.

8. Recently, the pharmaceuticals market has seen an expansion of the use of anti-inflammatory drugs for the treatment of many inflammatory conditions. The use of non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors, immune suppressants, and even monoclonal antibodies, such as REMICADE® (infliximab), have flourished for the treatment of diseases such as rheumatoid arthritis, inflammatory bowel disease, lupus, multiple sclerosis, psoriasis, and other inflammatory disorders. Severe adverse events have been associated with the use of all of these anti-inflammatory drugs to the extent that some have been withdrawn from the market (such as rofecoxib (VIOXX®)), while the use of others have been limited to short term administration (such as naproxen).

9. Treatment of periodontal disease requires administration of anti-inflammatory drugs to the patient for an extended period of time. The prolonged administration exposes the patient to a high degree of suffering from the above-mentioned adverse effects. It is generally believed among dentists that the risk of adverse events outweighs the therapeutic benefit of anti-inflammatory drugs.

10. Consequently, there are no anti-inflammatory drugs in the market for the treatment of periodontal disease. None of the currently available anti-inflammatory drugs are ever used for the treatment of periodontal disease. No dentist uses, or suggests the use of, anti-inflammatory medications for the treatment of periodontal disease. The standard of care for the

treatment of periodontal disease, as suggested by the American Dental Association, and as taught throughout dental schools in the United States, does not include the use of anti-inflammatory drugs.

11. The current thinking in the treatment of periodontal disease is summarized in Pihlstrom et al., Periodontol 2000, 2001, 25:37-58, "Periodontal risk assessment, diagnosis and treatment planning," a copy of which is attached as *Exhibit B*.

12. Today, periodontal disease is treated the way it was treated at the priority date of the above-captioned patent application, and at the time of publication of Pihlstrom et al. Treatment includes: systemic treatment, where the underlying systemic disease causing periodontal disease is targeted; hygienic treatment, where local causes of periodontal disease, including bacterial plaque and calculus are removed, and if necessary, antimicrobial agents, such as minocycline (ARESTIN®), are administered to kill the bacteria; corrective treatment, where procedures designed to correct the effect of periodontal disease are performed; and maintenance or supportive treatment.

13. The current treatment strategies or thinking do not include the use of anti-inflammatory drugs.

14. Because the use of anti-inflammatory drugs showed promise early on, the dental community has been in search of a suitable anti-inflammatory drug that can be effective in the treatment of periodontal disease without causing the typical adverse side effects common to other anti-inflammatory drugs. This search has proven elusive. No anti-inflammatory drug currently available on the market meets these requirements.

15. The use of cetylated fatty acids as anti-inflammatory formulations for the treatment of periodontal disease, in my opinion, is a major step forward. Cetylated fatty acids, to my knowledge, represent compounds that in animal studies have shown to provide the requisite anti-inflammatory effect without any adverse side effects.

16. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 1/8/08 By: /T. E. Van Dyke/
Thomas E. Van Dyke, DDS, PhD

EXHIBIT A

J Clin Periodontol 1993; 20: 457–464
Printed in Denmark.

JOURNAL OF
clinical periodontology
ISSN 0303-6979

The use of topical flurbiprofen as an adjunct to non-surgical management of periodontal disease

P. A. Heasman[1,2], D. K. Benn[3], P. J. Kelly[4], R. A. Seymour[2] and D. Aitken[2]

[1]Dental Research Center, UNC-Chapel Hill; [2]The Dental School, University of Newcastle upon Tyne, England; [3]Dental Radiology, Guy's Dental School, England; [4]Department of Medical Statistics, University of Newcastle upon Tyne, England

Heasman PA, Benn DK, Kelly PJ, Seymour RA and Aitken D: The use of topical flurbiprofen as an adjunct to non-surgical management of periodontal disease. J Clin Periodontol 1993; 20: 457–464. © Munksgaard, 1993.

Abstract. Non-steroidal anti-inflammatory drugs (NSAIDs) have been used as systemic and topical preparations to control chronic periodontal disease in both animal and clinical human trials. Equivocal findings have failed to confirm whether any one NSAID is particularly efficacious, although flurbiprofen appears to be one of the most promising. 49 patients were allocated at baseline to test (25) and control (24) groups in a 12-month, controlled clinical trial. The groups were of similar age and sex distributions. During the first 3 months, both groups were given oral hygiene instruction and received scaling and root planing. The test patients were prescribed a 1% w/w flurbiprofen toothpaste to use 2× daily for the entire 12 months. Control subjects were prescribed a placebo dentifrice. Plaque scores, bleeding scores, crevicular fluid flow, probing pocket depths and attachment levels were assessed at baseline and at 3, 6, 9 and 12 months. Radiographs were taken at baseline and 12 months using a modified intraoral, repositionable film holder. Both the flurbiprofen and placebo showed significant improvements in the clinical parameters over 12 months and there were no significant differences between the groups. Flurbiprofen-treated patients however, demonstrated a significantly greater proportion of sites (8.0%) with bone gain when compared to the placebo group (3.3%). There were no significant differences between the groups in the number of sites showing bone loss or no change. It is concluded that the 1% w/w flurbiprofen toothpaste exerts a small, yet significant effect on bone metabolism in the absence of any apparent effects on clinical parameters.

Key words: NSAIDs; flurbiprofen; periodontitis; digital analysis.

Accepted for publication 27 June 1992

The eicosanoids comprise a group of biochemical inflammatory mediators which is derived principally from the metabolism of arachidonic acid (ARA), an essential polyunsaturated fatty acid that is present in mammalian cell membrane phospholipid pools. There are two distinct ARA metabolic pathways. The action of the enzyme cyclooxygenase on ARA results in the synthesis of prostaglandins, thromboxanes and prostacyclin whereas the lipoxygenase pathway produces leukotrienes and hydroxyeicosatetraenoic acids. The detection of these mediators in periodontal tissues and crevicular fluid suggests that they play a significant role in the pathogenesis of periodontal disease (Goodson et al. 1974, El-Attar 1976, ElAttar & Lin 1980, 1981, Offenbacher et al. 1984, 1986, 1989).

Non-steroidal anti-inflammatory drugs inhibit the production of eicosanoids primarily by blocking the enzyme systems that convert arachidonic acid to the individual metabolites. Flurbiprofen (2-[2-fluoro-4-biphenyl] propionic acid) is a very potent cyclooxygenase inhibitor that has a wide range of pharmacological actions including the inhibition of polymorphonuclear leukocyte migration (Woodland et al. 1977a, 1977b), reduction of vascular permeability (Issekutz & Bhimji 1982) and inhibition of platelet aggregation (Nishizawa et al. 1973).

In a series of controlled longitudinal clinical trials on humans, flurbiprofen has been shown to be efficacious in the treatment of periodontal disease. From this standpoint, the principal effect of the NSAID appears to be the inhibition of bone loss (Jeffcoat et al. 1988, Williams et al. 1989, 1991, Ruttiman et al. 1991) although there is also evidence that associates a gain of clinical attachment with systemic flurbiprofen therapy (Abramson et al. 1990). Further, the beneficial actions

Table 1. Toothpaste study – entry profile

	Placebo	Flurbiprofen
no. subjects	24	25
males	17	16
age (years)	*41.83	*43.29
($m \pm 1$ s.d.)	(8.74)	(8.93)
no. withdrawals	3	1

* Student *t*-test ($p > 0.05$).

of flurbiprofen are lost when the therapy is withdrawn (Abramson et al. 1990, Williams et al. 1991). The majority of trials on humans were undertaken using systemic administration with doses of 50 mg 2 × daily. These would have been expected to sustain serum concentrations of about 2.5 μg/ml. Although this dose is well below those given for arthritic conditions, it is still likely to produce significant adverse reactions in about 20% of cases (Boots PLC Research Report). This % of adverse reactions in periodontal disease patients would be unacceptable, especially as the inflammatory disorder is not physically disabling. A topical route of administration of the drug may produce the same clinical effects, whilst minimizing systemic absorption, and is therefore to be considered preferable. Indeed, topical applications of NSAIDs using pastes, gels and biodegradable subgingival delivery systems have been shown to be efficacious in reducing attachment and bone loss in animal models (Vogel et al. 1986, Williams et al. 1988, Blodgett et al. 1989, Kornman et al. 1990, Yewey et al. 1991) but studies using such preparations in humans have yet to be published.

Material and Methods

49 adult patients, at least 18 years of age and suffering from moderate to advanced chronic adult periodontitis (AAP Types III and IV) were admitted to the study. All patients gave written consent prior to admission and approval of the study was obtained from the Regional Ethical Committee (Newcastle upon Tyne, UK). No subject was pregnant nor had a history of allergy to anti-inflammatory drugs, valvular heart disease, peptic ulceration, chronic dyspepsia or any other significant illness. No subject had a history of taking any NSAID (including aspirin) in the 12 months immediately prior to the trial.

Clinical examination

Clinical examinations were undertaken at baseline (0) and at 3, 6, 9 and 12 months. The clinical parameters were measured and recorded using a Periotron (IDE, New York, USA) and a Florida Probe (Florida Probe Company, Gainesville, FL USA) (Gibbs et al. 1988). The latter utilized the software package version 2.0 H. The Florida Probe hardware comprised:

IMB compatible COMCEN LX-10 laptop computer;
external RS232 computer interface and cable;
surge protector with master switch;
optical encoder, cable and foot control;
pressure-sensitive pocket probe, 0.4 mm tip diameter (20 gf);
pressure-sensitive attachment level disc probe, 0.4 mm tip diameter (20 gf).

Plaque scores

Plaque scores were recorded first. The presence or absence of a deposit was noted after staining the teeth with disclosing plaque dye (Oral B Laboratories, Bucks, England). Plaque was scored if a stained deposit was present at the cervical margin and if this deposit could be removed with a probe. The %s of surfaces with plaque were calculated.

Crevicular fluid flow

Plaque was carefully removed from the teeth with a piece of cotton wool and the surfaces were dried for 30 s with a continuous stream of moisture-free warm air. Teeth were isolated with cotton wool rolls and a periopaper was inserted atraumatically into each gingival crevice and left for 15 s. The periopaper was then transferred immediately into the jaws of a calibrated Peri-

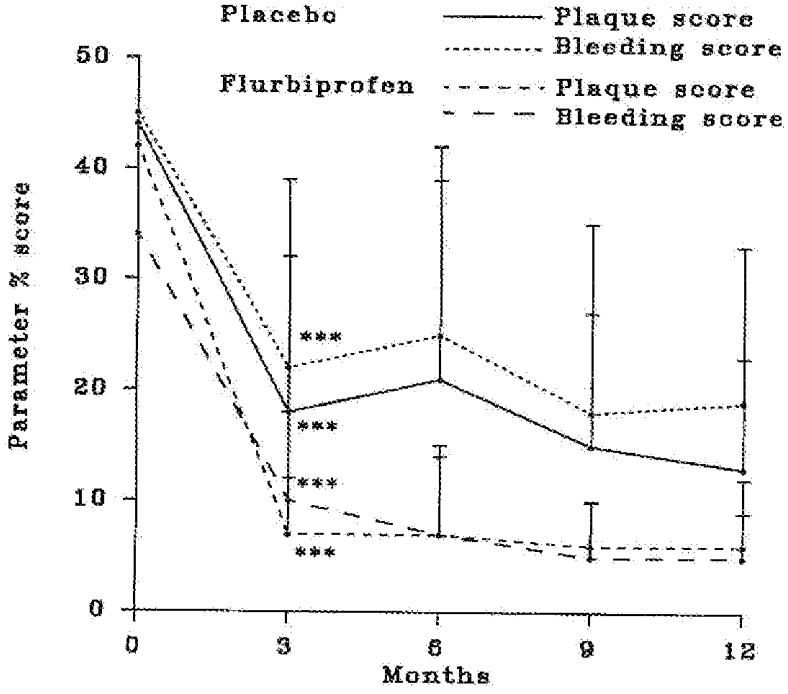

Fig. 1. Means (±1 standard deviations) of plaque and bleeding scores for flurbiprofen and placebo groups at baseline (0), 3, 6, 9 and 12 months.
There were no significant differences between treatments at any time point ($p > 0.05$).

Paired Student *t*-tests:		*t*	*p*
Plaque scores			
Placebo	0 × 3 months	7.879	< 0.001 (***)
	3 × 12 months	1.428	> 0.05 (NS)
Flurbiprofen	0 × 3 months	6.840	< 0.001 (***)
	3 × 12 months	0.710	> 0.05 (NS)
Bleeding scores			
Placebo	0 × 3 months	5.621	< 0.001 (***)
	3 × 12 months	2.835	< 0.05 (*)
Flurbiprofen	0 × 3 months	8.339	< 0.001 (***)
	3 × 12 months	0.119	> 0.05 (NS)

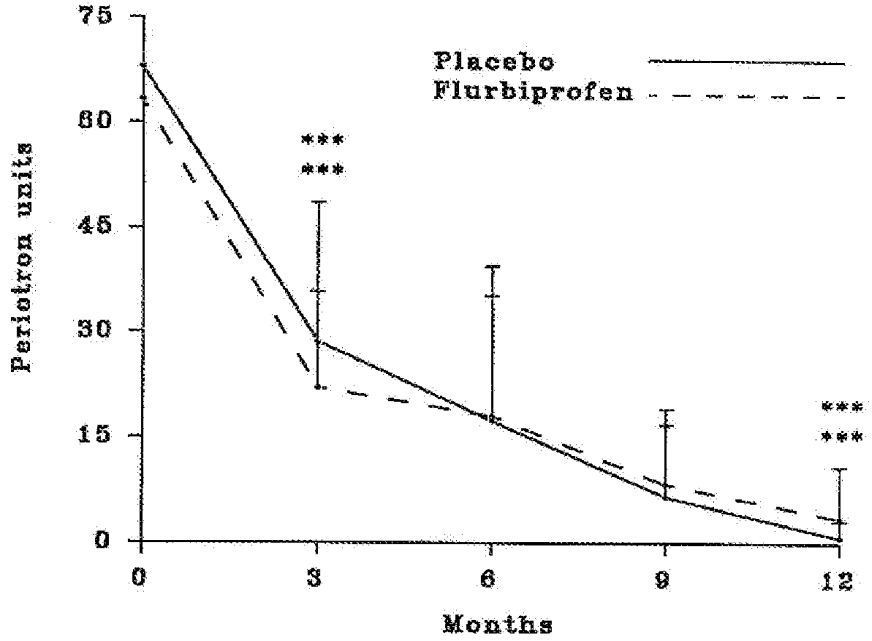

Fig. 2. Means (±1 standard deviations) of crevicular fluid flows (periotron units) in flurbiprofen and placebo groups at baseline (0), 3, 6, 9 and 12 months.

Paired Student *t*-tests:		*t*	*p*
GCF scores			
placebo	0 × 3 months	9.115	<0.001 (***)
	3 × 12 months	6.380	<0.001 (***)
flurbiprofen	0 × 3 months	8.257	<0.001 (***)
	3 × 12 months	6.179	<0.001 (***)

There were no significant differences between treatment at any time point ($p > 0.05$).

otron and the reading recorded. At each visit, a mean crevicular fluid flow was determined for each subject.

Probing pocket depths and attachment levels

The pocket depth and attachment measurements were recorded with the aid of a close-fitting hard acrylic onlay splint which provided reference points for the measurements. The placement of the probes was aided by steering grooves cut in the splints. The measurements were made to 0.1 mm.

Bleeding and pus scores

Bleeding and pus scores were made approximately 20 s after the corresponding probing pocket depths had been measured. If haemorrhage or a purulent exudate was noted subsequent to probing, a positive score was recorded. The proportion of bleeding and purulent sites as percentages of the total number of sites measured was calculated.

Sites for scoring

Crevicular fluid flow measurements were made on the mesiobuccal surfaces of the four first premolars (a second premolar was used when the first was missing). Probing pocket depths, attachment measurements and dichotomous bleeding, pus and plaque scores were recorded at 6 points (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual) around the 2 premolar and first permanent molar teeth in each quadrant. When the 1st permanent molar or 2nd premolar was absent, the 2nd molar was used. When a 1st premolar was absent, the canine was charted.

The clinical data were recorded immediately on a double-density, double-sided hard disc (Nashua MF-2DD, 1.0 MB capacity). The pocket probing depth and attachment level data were transferred to the University Mainframe computer for statistical analysis.

Radiographic examination

Periapical radiographs were taken at baseline and at 12 months using a bitewing, stentless, repositionable film holder (Benn 1988, Whaites 1992). The holder was modified from the conventional bitewing position by holding the film 5 mm more apically. In this way, approximately 9 mm of alveolar bone crest were visible in one jaw as opposed to about 4 mm in both jaws in a normal bitewing view. The modified bitewing projection was selected since this is likely to produce less distortion of the alveolar crest than a paralleling periapical projection (Hausmann et al. 1989).

The radiographic hardware comprised a GE 100 X-ray set (General Electric, USA) with a focal spot of 1.0 mm and intrinsic filtration equivalent to 2.7 mm of aluminum at 100 kVp. Exposures were made at 65 kVp, 10 mA and 0.4 s with a focus to film distance of approximately 270 mm. All films were exposed by the same operator (DA). A number 2 sized ultraspeed intraoral film (Kodak, Rochester, NY) was exposed for each posterior region attempting to cover from the distal of the canine to the distal of the second molar in one jaw. After each exposure, the anatomical coordinates were recorded (Benn 1988). In all, 4 intraoral films were exposed for each patient at the start of the study. After 12 months, the film holders were repositioned using the recorded anatomical coordinates and a further 4 films exposed. All films were hand processed by one operator (DKB) according to the manufacturers instructions (Kodak, Rochester, NY).

Computer system

CEJ to alveolar bone crest measurements were made using digital images and stored regions of interest (Benn 1992). Previous evaluation of the method had shown an intra-examiner reliability of ±0.15 mm for CEJ to crest measurements (Benn 1992). The crest reference site was identified either as the most coronal portion of the periodontal ligament space of constant width, the point where the crest image crossed the root if a periodontal ligament space was not visible or, the point at which bony trabeculae became visible at the base of an infrabony lesion (Benn 1992).

Each image pixel represented a region of 0.5 mm by 0.5 mm on the film. Using a mouse, the CEJ and crest reference points were selected twice for each site in the initial and then the final films of the pairs. The software automatically calculated the reliability of the measurements at 90%, 95%, 96%, 98% and 99% confidence levels for all sites (Benn 1992). Alveolar bone height changes with a measurement reliability of less

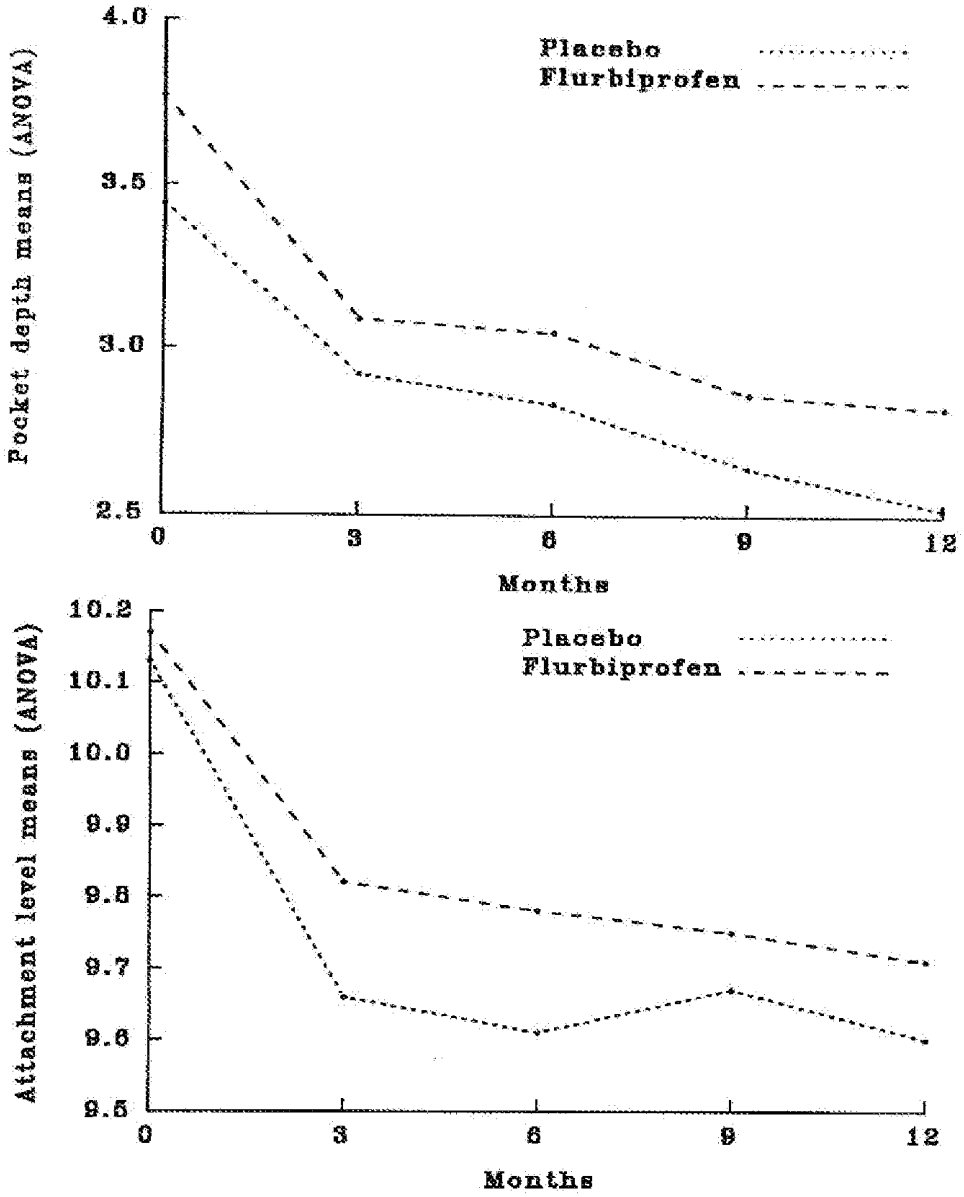

Fig. 3. Pocket depth and attachment level means data used for ANOVA treatments with the unit of analysis being the site around the tooth. Attachment measurements were made from the base of the disc on the Florida Probe to the base of the pocket.

than 95% were assumed to be due to measurement error and assigned to the no change group of sites. All measurements were made by one operator (DKB).

Validation of sequential films of comparison

No attempt was made to digitally alter the contrast of the film images. If a CEJ or crestal site became very dark after digitization, it was excluded from the survey. Geometry changes between serial films were detected by alteration of (1) the periodontal ligament space width, (2) the extent of the approximal tooth surface overlaps, and (3) the parallax shift of premolar cusps in either the horizontal or vertical planes. When approximal surface overlap changes were greater than 0.1 mm, the film pair was excluded. The other 2 classes of change were judged subjectively.

Treatment procedures

At baseline, each patient was randomly allocated to 1 of 2 groups:

Flurbiprofen: (25 patients) Each patient was given 10 pump pack tubes of 1% w/w flurbiprofen toothpaste*. One tube contained 140 g of paste. The patients were instructed to use approximately 2 cm of toothpaste when brushing each morning and evening for 12 months.

Placebo: (24 patients) Each patient was given 10 pump pack tubes of a placebo toothpaste*. One tube contained 140 g of paste and the same instructions for brushing were given.

Apart from the inclusion of flurbiprofen, the two toothpastes were of identical composition. For the duration of the trial, double blind conditions were observed. All patients were asked to report immediately any adverse reactions which they believe may have arisen from using the toothpastes.

Non-surgical treatment regimens were implemented on both flurbiprofen and placebo patients. Between baseline and the 3-month appointments, a regimen of oral hygiene instruction was enforced. The Bass toothbrushing technique was demonstrated in addition to the use of dental floss and mini-interdental (interproximal) brushes. Full mouth scaling and root planing was undertaken under local anesthesia and operating upon one quadrant per visit. Any local plaque retentive factors such as overhanging or poorly contoured restortions were eliminated.

Between the 6 and 9 month appointments, oral hygiene instructions were reinforced according to individual patient needs. Between the 9- and 12-month appointments, a 2nd scaling was performed primarily to remove any reformed supragingival calculus. Oral hygiene instructions were again reinforced according to requirements. When the 12-month examinations had been completed, a final scaling was undertaken and the patients referred back to their general practitioners with instructions for routine maintenance therapy. All measurements and the treatment procedures were undertaken by one operator (PAH).

Statistical analysis

The means of the crevicular fluid flow, plaque and bleeding score data were analysed using the Student *t*-test. The probing pocket depth and attachment level data were analysed on an individual site basis using hierarchical analysis of variance. The ANOVA and *t*-test analyses were carried out using the Genstat 5.0 and Minitab programs respectively on the University Mainframe

* Boots PIC, Nottingham, England.

Table 2. Analysis of variance of data with unit as the tooth site

	df	Ssq	MsQ	VR	F
Between groups PD					
treatment	1	2.239	2.239	2.19	0.146 NS
residual	43	4.389	1.021		
Within groups PD					
time	4	1.824	4.561	576.75	<0.001
treatment × time	4	1.402	3.505	4.43	0.001
residual	11545	9.130	0.791		
Between groups PD					
treatment	1	4.555	4.555	0.08	0.785 NS
residual	42	2.540	6.046		
Within groups PD					
time	4	5.228	1.307	163.52	<0.001
treatment × time	4	8.859	2.215	2.77	0.026
residual	11615	9.284	0.799		

A summary of the ANOVA treatments used in the analyses of pocket depths and attachment levels.

flow rates were significantly reduced ($p<0.001$) in each group and after 12 months the flow rates were reduced further when compared to the 3 month values ($p<0.001$). There were no significant differences between the groups at any time point ($p<0.05$).

Probing pocket depths and attachment levels. The graphical representations of the mean data used in the ANOVA treatments of probing pocket depths and attachment levels are shown in Fig. 3. The detailed statistical analysis is given in Table 2. Pocket depths and attachment levels improved significantly over the 12 months of the study but there were no significant differences between the groups.

computer. The analysis of bone changes was undertaken using a comparison of 2 proportions at an individual site level.

Results

Entry profile data

The subject data for the 2 groups are presented in Table 1. 17 of the 25 flurbiprofen patients were male compared to 16 of the placebo group. There was no significant difference in the mean ages of the groups.

After 12 months of the trial, 4 patients had withdrawn (3 placebo) because they were unable to attend the interim appointments. Data of 9 patients were not recorded for a single visit for reasons of non-attendance. The data of the remaining 4 visits in each case (always including the first and the last) were included in the overall statistical analysis. None of the patients complained of symptoms or presented with adverse effects as a consequence of using the toothpaste.

Clinical parameters

Plaque scores. The means and standard deviations of the plaque scores for the groups at each time point are presented graphically in Fig. 1. At baseline, the mean plaque scores were almost identical in each group (45% flurbiprofen and 44% placebo). In both groups, the scores were reduced significantly ($p<0.001$) at 3 months. The scores were then maintained at between 13 and 25% for the remaining 9 months of the trial during which they did not differ significantly from the 3 month values. There were no significant differences between the groups at any time point ($p>0.05$).

Bleeding and pus scores. Only a very small number of pockets exuded a purulent exudate throughout the trial and those that did also demonstrated profuse bleeding. For this reason, the sites which exuded pus were neither charted nor analysed separately from the bleeding scores. The means and standard deviations of the bleeding scores are also presented in Fig. 1. The baseline mean bleeding scores were 42% and 34% for the flurbiprofen and placebo groups, respectively. At 3 months, both groups showed significant reductions in scores ($p<0.001$) to 7% and 10%, respectively. The scores in flurbiprofen group varied by only 1% at future time points whereas the scores of the placebo group showed a further significant reduction by the end of the trial.

Crevicular fluid flow. The mean crevicular fluid flow measurements at each time point are presented graphically in Fig. 2 from which it can be seen that the group means at each time point are very similar. At the 3-month visits, the

Radiographic findings

Of the 49 patients who were enrolled at baseline, 8 patients were unable to tolerate the radiographic holder. Therefore, 41 patients completed the serial radiographic examination and a total of 910 sites were available for analysis. However, due to adverse optical density or irradiation geometry changes, only 419 (46% of the total) sites were measured. Of these, 339 (81%) showed no change, 25 (6%) bone gain and 55 (13%) bone loss. 6 of the bone gain sites were in the placebo group and 19 in the flurbiprofen group. Of the no change sites, 155 were placebo and 184 flurbiprofen. By comparison of 2 proportions, the null hypothesis was rejected at the 5% level for bone gain. However, there was no statistically significant difference for bone loss between the 2 groups.

An analysis by magnitude of change for all sites which altered by loss or gain (80) showed that 19 (24%) were <0.5 mm, 43 (54%) changed between 0.5–1.0 mm, and 18 (22%) were ≥1.0 mm. 62 (78%) sites changed by <1.0 mm.

Table 3. Analysis of radiographic alveolar bone changes following the use of a placebo or flurbiprofen-containing toothpaste over a 12-month period

	Placebo	Flurbiprofen
no. sites available on radiographs	367	543
no. sites measured	183	236
no. sites showing *GAIN*	6 [3.3]	19 [8.0]**
no. sites showing *LOSS*	22 [12.0]	33 [14.0]*
no. sites with *NO CHANGES*	155 [84.7]	184 [78.0]

Figures in parentheses given the proportions of the sites with bone gain, loss or no change as percentages of the number of sites measured.

* d/SEd = 0.59 [<1.96] NS.

** d/SEd = 2.17 [>1.96] difference highly significant.

Discussion

The aim of this controlled study was to assess the efficacy of a flurbiprofen-containing toothpaste when applied as an adjunctive measure in the non-surgical management of chronic adult periodontal disease. At baseline, the 2 groups were closely matched for both age and sex. The overall attendance of the patients was excellent and 92% of those enrolled went on to complete the trial. The compliance of the patients with oral hygiene procedures was also high, as evidenced by the rapid and maintained reduction in plaque scores. The compliance with the use of the prescribed toothpastes was assessed only by verbal communication, but all of the patients who completed the trial claimed continued use of the dentrifices after 12 months.

In both the flurbiprofen and placebo-treated groups, there were significant reductions in plaque and bleeding scores, crevicular fluid flow and probing depths following non-surgical management (0–3 months). There were also significant improvements in attachment levels in both groups (Table 3). However, the inter-group analyses failed to detect any significant differences between the treatments. These observations were not totally unexpected. The implementation of a non-surgical treatment regimen on compliant and motivated individuals can have a powerful effect in reducing the clinical expression and progression of periodontal disease (Badersten 1981, 1984). The additional actions of a topical preparation would therefore need to produce significant clinical effects if they are to be detected in a trial of this design. In this respect, the findings of our study are consistent with those of Williams and coworkers who reported that systemic flurbiprofen (0.02 mg/kg/day) did not affect severe, established gingival inflammation in old beagle dogs when given for 12 months as an adjunct to non-surgical therapy (Williams et al. 1985). Further, in a controlled human study, systemic flurbiprofen was given as an adjunct to subgingival scaling and was associated with a reduction in gingival inflammation (Williams et al. 1989). However, in comparison with our study, a much larger dose of flurbiprofen (50 mg b.d.) was prescribed (in tablet form) for twice the period of time (24 months) before the effect was observed. Conversely, in a study on monkeys, flurbiprofen therapies of 0.27 mg/kg/day and 7.1 mg/kg/day were found to significantly inhibit attachment loss ($p<0.05$), gingival redness ($p<0.05$) and bleeding on probing ($p<0.05$) in a non dose-dependent manner over 6 months (Offenbacher et al. 1987). The trial design however, was such that systemic flurbiprofen was the only treatment regimen used and this may have permitted a more critical assessment of changes of clinical disease expression.

Perhaps the most exciting aspect of flurbiprofen therapy in the management of periodontal disease is associated with its effect in inhibiting bone loss. This observation has been widely demonstrated in both animal and human disease models (for review, see Offenbacher et al. (1992a)). In beagle dogs, e.g., Jeffcoat et al. (1986) showed that systemic flurbiprofen reduced the uptake of a bone-seeking radiopharmaceutical compound and concluded that there was both a reduction in the number of active sites and an increase in the number of quiescent sites in NSAID-treated animals. In the present study, there appeared to be no difference between the groups in the number of sites that exhibited bone loss, although the assessment of change using radiographs is less sensitive than the method used by Jeffcoat et al. (1986). However, a difference was apparent in the number of sites demonstrating bone gain which was proportionally larger in the flurbiprofen group. This difference, although relatively small, was statistically significant and suggested that the topical application of flurbiprofen in humans may have similar effects to those observed by other workers using systemic NSAIDs.

The incorporation of flurbiprofen into a toothpaste preparation was undertaken for several reasons. There is an increasing body of evidence that suggests that topical applications of NSAIDs can also reduce bone resorption when applied intraorally (Williams et al. 1988, Kornman et al. 1990, Offenbacher et al. 1992b). A topical application however, does not wholly exclude systemic absorption after swallowing as suggested by Williams et al. (1988) and indeed there are also studies that have detected plasma levels of flurbiprofen after use of both rinsing solutions and toothpaste (Stalker et al. 1987, Heasman 1992). A topical preparation should also reduce the number of adverse reactions to any NSAID as these are predominantly associated with gastrointestinal irritation. It is therefore of interest to note that the patients in our study experienced neither gastrointestinal problems nor oral reactions to the toothpaste. We also suggest that patients may be more compliant with the daily use of a dentifrice rather than with frequent and long-term dosing with systemic NSAIDs. Indeed, in a recent report, Williams et al. (1991) discovered a non-compliant subpopulation of patients within the cohort of subjects enrolled in their 3-year clinical trial using systemic flurbiprofen therapy. In studies of this nature, compliance with the NSAID therapy is essential as attenuation of dosing is associated with a loss of beneficial clinical effect (Williams et al. 1989, 1991).

Finally, in view of both the complete absence of adverse reactions in our patients and the small, yet significant effect of the flurbiprofen toothpaste on bone changes, we suggest that future research may be directed toward increasing the dose of flurbiprofen in the toothpaste and perhaps increasing the frequency of application. It is conceivable that the therapeutic efficacy of flurbiprofen to prevent bone loss may be likened to the effect of fluoride on caries and ultimately periodontal disease may also be controlled successfully by the addition of specific, active agents to toothpaste formulations.

Acknowledgements

This research study was supported by The Upjohn Company. The authors thank Betsey Warhurst for preparing the manuscript.

Zusammenfassung

Lokale Applikation von Flurbiprofen zur Ergänzung nicht-chirurgischer Behandlung der Parodontalkrankheit

Nicht-steroide, entzündungshemmende Medikamente (NSAIDs = non-steroidal-anti-inflammatory drugs) sind sowohl bei Tierversuchen als auch bei klinischen Untersuchungen am Menschen als systemische und lokale Präparate zur Kontrolle chronisch parodontaler Erkrankungen angewandt worden. Die keinesfalls eindeutigen Ergebnisse haben nicht bestätigen können, daß irgendein NSAID besonders wirksam ist, obwohl Flurbiprofen eines der vielversprechendsten zu sein scheint. 49 Patienten wurden anläßlich der Eingangsuntersuchung zu einem 12 Monate langen, kontrollierten klinischen Versuch in Test- (25) und Kontrollgruppen (24) eingeteilt. Die Alters- und Geschlechtsverteilungen der Versuchsgruppen waren etwa gleich. Während der ersten 3 Monate wurden beide Gruppen

in der Praxis der Mundhygiene instruiert und mit Zahnsteinentfernung und Wurzelglättung behandelt. Den Testpatienten wurde eine 1%ige w/w Flurbiprofen-Zahnpasta zu zweimal täglichem, 12 Monate langen Gebrauch verordnet. Den Kontrollpatienten wurde eine Plazebo-Zahnpasta ausgehändigt. Die Beurteilungseinheiten für Plaque, Zahnfleischbluten, Zahnfleischsekretfluß, sondierte Taschentiefen und Attachmentniveaus wurden anläßlich der Eingangsuntersuchung (baseline) und nach 3, 6, 9 und 12 Monaten festgestellt. Bei der Eingangsuntersuchung und nach 12 Monaten wurden Röntgenaufnahmen mit Hilfe eines modifizierten intraoralen, auswechselbaren Filmhalters hergestellt. Sowohl bei den Flurbiprofen- als auch bein den Plazebogruppen wurden während der 12 Versuchsmonate signifikante Verbesserungen der klinischen Parameter beobachtet, nicht jedoch zwischen den Gruppen. Bei den mit Flurbiprofen behandelten Patienten wurde jedoch, im Vergleich zu der Plazebogruppe (3.3%), ein signifikant häufigeres Vorkommen von Stellen mit Knochengewinn (8.0%) konstatiert. Hinsichtlich der Anzahl von Stellen mit Knochenverlust oder keiner Veränderung, lagen zwischen den Gruppen keine signifikanten Unterschiede vor. Es wird gefolgert, daß die 1%ige w/w Flurbiprofenzahnpasta den Metabolismus des Knochens gering aber signifikant beeinflußt, obwohl keine offensichtliche Beeinflussung der klinischen Parameter beobachtet wird.

Résumé

Utilisation de flurbiprofène topique en tant que complément du traitement non chirurgical de la maladie parodontale

Les drogues anti-inflammatoires non stéroïdales (NSAID) ont été utilisées en tant que préparation topique et systémique pour contrôler la maladie parodontale chronique dans des expériences cliniques chez l'animal comme chez l'homme. Des découvertes équivoques n'ont pu confirmer ou infirmer si l'une ou l'autre des NSAID est particulièrement efficace et ce, alors que le flurbiprofène semblait un des plus prometteurs. 49 patients ont été répartis en 25 dans le groupe test et 24 dans le groupe contrôle pour cette expérience clinique contrôlée de 12 mois. Les groupes avaient une distribution semblable en ce qui concerne l'âge et le sexe. Durant les 3 premiers mois les deux groupes ont reçu une instruction en hygiène buccale et subi un détartrage et lissage radiculaire. Un dentifrice de flurbiprofène 1% w/w a été prescrit aux patients tests afin qu'ils l'utilisent deux fois par jour pendant 12 mois. Les sujets du groupe contrôle ont reçu un dentifrice placebo. Les scores de plaque, de saignement et de flux créviculaire ainsi que des profondeurs de poches et des niveaux d'attache ont été relevés lors de l'examen initial et après 3, 6, 9 et 12 mois. Des radiographies ont été prises lors de l'examen initial et 12 mois après en utilisant une méthode permettant le repositionnement du porte film. Aucune différence n'a été remarquée entre les groupes qui, tous deux, accusaient des améliorations significatives des paramètres cliniques. Cependant les patients tests avaient une proportion significativement plus importante de sites avec gain osseux (8.0%) lorsqu'on les comparait à ceux du groupe placebo (3.3%). Il n'y avait aucune différence entre les groupes dans le nombre de sites montrant une perte osseuse ou aucun changement. Le dentifrice au flurbiprofène exerce donc un effet, petit mais significatif, sur le métabolisme osseux en l'absence de tout paramètre clinique apparent.

References

Abramson, M. M., Wolff, L. F., Aeppli, D. M., Hardie, N. A. & Friedman, H. (1990) Flurbiprofen effect on prostaglandin/thromboxane levels in human crevicular fluid. *Journal of Dental Research* **69**, 219.

Badersten, A., Nilveus, R. & Egelberg, J. (1981) Effect of non-surgical periodontal therapy (I). Moderately advanced periodontitis. *Journal of Clinical Periodontology* **8**, 57–72.

Badersten, A., Nilveus, R. & Egelberg, J. (1984) Effect of non-surgical periodontal therapy (II). Severely advanced periodontitis. *Journal of Clinical Periodontology* **11**, 63–76.

Benn, D. K. (1988) New methods for diagnosing approximal caries and alveolar bone loss from bitewing radiographs. Proceedings of the British Society of Dental and Maxillofacial Surgery. *Maxillofacial Radiology* **3**, 13–16.

Benn, D. K. (1992) A computer-assisted method for making linear radiographic measurements using stored regions of interest. *Journal of Clinical Periodontology* **19**, 441–448.

Blodgett, R. F., Brunsvold, M. A., Holt, S. C. & Kornman, K. S. (1989) The gingival crevicular PMN response in monkeys receiving NSAIDs. *Journal of Dental Research* **68**, 242, abstr. 490.

Boots, P. L. C. (1983) Research Department Report.

ElAttar, T. M. A. (1976) PGE in human gingiva in health and disease and its stimulation by female sex steroids. *Prostaglandins* **11**, 331–342.

ElAttar, T. M. A. & Lin, H. S. (1980) Cyclic AMP and prostaglandins in periodontal disease. In: *Advances in prostaglandin and thromboxane research*, eds. Samuelsson, B., Ramwell, P. W. & Paoletti, R., pp. (8) 1739–1740. Raven Press, NY.

ElAttar, T. M. A. & Lin, H. S. (1981) Prostaglandins in gingiva of patients with periodontal disease. *Journal of Periodontology* **52**, 16–19.

Gibbs, C. H., Hirschfeld, J. W., Lee, J. G., Low, S. B., Magnusson, I., Thousand, R. R., Yerneni, P. & Clark, W. B. (1988) Description and clinical evaluation of the new computerized periodontal probe – the Florida probe. *Journal of Clinical Periodontology* **15**, 137–144.

Goodson, J. M., Dewhirst, F. E. & Brunetti, A. (1974) Prostaglandin E levels and human periodontal disease. *Prostaglandins* **6**, 81–85.

Hausmann, E., Allen, K., Christersson, L. & Genco, R. (1989) Effect of X-ray beam vertical angulation on radiographic alveolar crest level measurement. *Journal of Periodontal Research* **24**, 8–19.

Heasman, P. A. (1992) *The effects of non-steroidal anti-inflammatory drugs on periodontal disease*. PhD thesis, University of Newcastle upon Tyne.

Issekutz, A. C. & Bhimji, S. (1982) The effect of non-steroidal anti-inflammatory agents on *E. coli* induced inflammation. *Immunopharmacology* **4**, 11–22.

Jeffcoat, M. K., Williams, R. C., Wechter, W. J., Johnson, H. G., Kaplan, M. L., Gandrup, J. S. & Goldhaber, P. (1986) Flurbiprofen treatment of periodontal disease in beagles. *Journal of Periodontal Research* **21**, 624–633.

Jeffcoat, M. K., Williams, R. C., Reddy, M. S., Rolla, A., English, R. & Goldhaber, P. (1988) Short-term flurbiprofen administration for the treatment of refractory periodontitis in humans. *Journal of Dental Research* **67**, 371, abstr. 2063.

Kornman, K. S., Blodgett, R. F., Brunsvold, M. & Holt, S. C. (1990) Effects of topical applications of meclofenamic acid and ibuprofen on bone loss, subgingival microbiota and gingival PMN response in the primate *Macaca fascicularis*. *Journal of Periodontal Research* **25**, 300–307.

Nishizawa, E. E., Wynalda, D. J., Suydam, E. D. & Moloney, B. A. (1973) Flurbiprofen, a new potent inhibitor of platelet aggregation. *Thrombosis Research* **3**, 577–588.

Offenbacher, S., Odle, B. M., Gray, R. C. & Van Dyke, T. E. (1984) Crevicular fluid prostaglandin E levels as a measure of the periodontal disease status of adult and juvenile periodontitis patients. *Journal of Periodontal Research* **19**, 1–13.

Offenbacher, S., Odle, B. M. & Van Dyke, T. E. (1986) The use of crevicular fluid PGE_2 levels as a predictor of periodontal attachment loss. *Journal of Periodontal Research* **21**, 101–112.

Offenbacher, S., Braswell, L. D., Loos, A. S., Johnson, H., Hall, C., McClure, H., Orkin, J. L. & Stobert, E. A. (1987) Effects of flurbiprofen on the progression of periodontitis in *Macaca mulatta*. *Journal of Periodontal Research* **22**, 473–481.

Offenbacher, S., Odle, B. M., Braswell, L. D., Johnson, H. G., Hall, C. M., McClure, H., Orkin, J. L., Strobert, E. A. & Green, M. E. (1989) Changes in cyclooxygenase metabolites in experimental periodontitis in *Macaca mulatta*. *Journal of Periodontal Research* **24**, 63–74.

Offenbacher, S., Heasman, P. A. & Collins, J. G. (1992a) Modulation of host PGE_2 secretion as a determinant of periodontal disease expression. *Journal of Periodontology*, in press.

Offenbacher, S., Williams, R. C., Jeffcoat, M. K. (1992b) Effects of NSAIDs on beagle crevicular fluid cyclooxygenase metabolites and periodontal bone loss. *Journal of Periodontal Research*, in press.

Ruttiman, U. E., de Valk, S. A., Engelke, W. H., Wright, N. E. & Cain, J. L. (1991) Effect of flurbiprofen on alveolar bone loss assessed by subtraction radiography. *Journal of Dental Research* **70**, 468, abstr. 1616.

Stalker, D. J., Pollock, S. R. & Albert, K. S. (1987) The *in vivo* assessment of buccal absorption of flurbiprofen. *American Pharmaceutical Science Association*. Annual Meeting, abstr. 57.

Vogel, R. I., Schneider, L. & Goteiner, D. (1986) The effects of a topically active non-steroidal anti-inflammatory drug on ligature-induced periodontal disease in the squirrel monkey. *Journal of Clinical Periodontology* **13**, 139–144.

Whaites, E. In: *Essentials of dental radiography and radiology*, p. 84. Edinburgh: Churchill Livingstone.

Williams, R. C. Jeffcoat, M. K., Kaplan, M. L., Goldhaber, P., Johnson, H. G. & Wechter, W. J. (1985) Flurbiprofen – A potent inhibitor of alveolar bone resorption in beagles. *Science* **227**, 640–642.

Williams, R. C., Jeffcoat, M. K., Howell, T. H., Hall, C. M., Johnson, H. G., Reddy, M. S. & Goldhaber, P. (1988) Topical flurbiprofen treatment of periodontitis in beagles. *Journal of Periodontal Research* **23**, 166–169.

Williams, R. C., Jeffcoat, M. K., Howell, T. H., Rolla, A., Stubbs, D., Teoh, K. W., Reddy, M. S. & Goldhaber, P. (1989) Altering the progression of human alveolar bone loss with the non-steroidal anti-inflammatory drug flurbiprofen. *Journal of Periodontology* **60**, 485–490.

Williams, R. C., Jeffcoat, M. K., Howell, T. H., Paquette, D., Rolla, A., Reddy, M. A. & Goldhaber, P. (1991) Three year trial of flurbiprofen treatment in humans: Post-treatment period. *Journal of Dental Research* **70**, 468, abstr. 1617.

Woodland, J., Vernon-Roberts, B. & Swettenham, K. V. (1977a) Comparison of the effects of alclofenac, flurbiprofen and prednisolone on acute inflammatory effects response in the rat. *Annals of Rheumatoid Disorders* **36**, 160–165.

Woodland, J., Currey, H. L. & Vernon-Roberts, B. (1977b) The effects of anti-inflammatory and anti-rheumatic drugs on inflammation in the rat. *Current Medical Research and Opinions* **5**, 3–10.

Yewey, G. L., Tipton, A. J., Dunn, R. L., Menardi, E. M., McEvoy, R. M., Jensen, J. A., Southard, G. L. & Polson, A. M. (1991) Evaluation of biodegradeable subgingival delivery system for flurbiprofen. *Journal of Dental Research* **70**, 324, abstr. 468.

Address:

P. A. Heasman
Department of Operative Dentistry
The Dental School,
Framlington Place,
Newcastle-upon-Tyne,
NE2 4BW
England

EXHIBIT B

Periodontology 2000, Vol. 25, 2001, 37–58
Printed in Denmark · All rights reserved

PERIODONTOLOGY 2000
ISSN 0906-6713

Periodontal risk assessment, diagnosis and treatment planning

BRUCE L. PIHLSTROM

In today's health- and cost-conscious environment, it is essential that rational and cost-effective decisions be made for prevention and treatment of the periodontal diseases. The prevention and treatment of disease is based on accurate diagnosis, reduction or elimination of causative agents, risk management and correction of the harmful effects of disease. Since there are many types of periodontal diseases that require different treatment methods, it is critical that an accurate diagnosis be established. The purpose of this chapter is to provide the general dental practitioner with an overview and update of risk assessment, diagnostic methods and treatment planning for patients with various types of periodontal diseases.

Periodontal risk assessment

Risk assessment is a way of examining risks so that they may be avoided, reduced, or managed (119). Risk can be identified in terms of risk factors, risk indicators, or risk predictors (14). A risk factor is thought to be causal for a disease. As such, it should satisfy two criteria: 1) it is biologically plausible as a causal agent for disease and, 2) it has been shown to precede the development of disease in prospective (forward design) clinical studies. Smoking is an example of a risk factor for periodontal disease, since there are a number of biologically plausible explanations for it as a causative agent for periodontal disease, and prospective clinical studies have shown that smokers are more likely to develop periodontitis than nonsmokers. A risk indicator is a factor that is biologically plausible as a causative agent for disease but has only been shown to be associated with disease in cross-sectional studies. Some risk indicators may be proven to be risk factors if prospective studies are able to confirm that they precede the development of disease. An example of a risk indicator of periodontal disease is the presence of herpesviruses in subgingival plaque. There is a biologically plausible explanation why herpesviruses may be causally related to periodontal disease, but so far, the evidence of association with disease is based on cross-sectional studies (31). A risk predictor is a factor that has no current biological plausibility as a causative agent but has been associated with disease on a cross-sectional or longitudinal basis. Risk predictors may be either markers of disease or other historical measures of disease (14). Examples are the number of missing teeth or past evidence of periodontal disease. The number of missing teeth is a risk predictor for disease, but has little or no biological plausibility as a causative agent for periodontitis.

The risk for disease is often quantified using relative risks and/or odds ratios. Relative risk is the probability of developing disease if one is exposed to a given factor compared with the probability of developing the disease if one is not exposed to the factor. In the example given in Table 1, smokers and nonsmokers were matched for age, sex, plaque and calculus. The relative risk for smokers to have deeper pockets was 15/63÷7/126 or about 4.3. This means that smokers were 4.3 times more likely to have deeper pockets than nonsmokers. An odds ratio is defined as the odds of having disease if one is exposed to a risk factor compared with the odds of having the disease if one is not exposed to the same factor. The odds ratio for smokers to have

Table 1. Data from a cross-sectional study of periodontal disease

Patient type	Patients with deep average probing depth	Patients with shallow average probing depth	Total number of patients
Smokers	15	48	63
Nonsmokers	7	119	126

Relative risk of disease in smokers=15/63÷7/126=4.3; odds ratio of disease between smokers and nonsmokers=15/48÷7/119=5.3.

deeper pockets versus nonsmokers in Table 1 is 5.3 (15/48÷7/119). The odds ratio and relative risk are similar when the prevalence of disease is low, but the values diverge as disease prevalence increases. While both relative risk and odds ratios are used to quantify risk, relative risk is generally more intuitively meaningful for clinicians.

Risk factors and indicators for periodontal disease

During the past decade, many risk factors have been identified for the periodontal diseases (36, 39, 92, 97). A comprehensive discussion of all risk factors for each of the periodontal diseases is beyond the scope of this chapter, and attention will be focused on plaque-associated gingivitis and chronic periodontitis in adults. Besides the association of periodontal disease with the local factors of bacterial plaque and dental calculus, open proximal tooth contacts and food impaction have been associated with increased loss of attachment, decreased crestal bone support and increased probing depth (53, 62, 66). Moreover, traumatic occlusion has been associated with decreased crestal bone height (100), and the presence of a parafunctional habit without the use of a nightguard has been associated with a poorer periodontal prognosis following periodontal therapy (81). Specific teeth also appear to have a poorer prognosis following periodontal therapy. During the maintenance phase following active periodontal therapy, molar teeth (particularly maxillary second molars) are among the teeth most frequently lost, while mandibular canines and first premolars have the highest retention rate (42, 58).

Smoking has been confirmed as a true risk factor for periodontitis in longitudinal studies (15, 21, 24, 25) with odds ratios for periodontitis in the range of 2.0 to 7.0 (20, 39). Furthermore, heavy smokers have odds ratios that are over two times that of light smokers for loss of attachment (4.75 versus 2.05) and bone support (7.28 versus 3.25) (46, 47). Smokers are also at increased risk for tooth loss following periodontal therapy (81).

Diabetes is also a true risk factor for periodontitis. Type 1 diabetes is caused by an absolute insulin deficiency resulting from destruction of pancreatic beta cells. In contrast, type 2 diabetes is caused by impaired insulin function and a relative insulin deficiency (83). Type 1 diabetes usually has its onset in childhood, while type 2 diabetes occurs in adulthood and is often associated with obesity. Diabetes has a variety of complications including retinopathy, nephropathy, neuropathy, vascular disease and altered wound healing. It is clinically associated with increased susceptibility to infection and individuals with both types of diabetes are at increased risk for periodontal disease. Poor diabetic control in the presence of calculus is associated with an increased frequency of probing depths ≥4 mm (113). Furthermore, many longitudinal and cross-sectional studies have documented an association between diabetes or poor diabetic control and attachment or periodontal bone loss (odds ratio=2.6 to 11.4) (29, 88, 107, 109, 111–113).

Cross-sectional studies have established several risk indicators that are associated with periodontitis. The presence of *Porphyromonas gingivalis, Bacteroides forsythus, Prevotella intermedia* and *Fusobacterium nucleatum*, being male, and older age are associated with periodontitis (1, 39, 92). Moreover, Epstein-Barr virus type 1 and human cytomegalovirus have a positive association with several periodontal bacteria and with periodontitis (31). A longitudinal study of up to seven years indicated that smoking, *P. gingivalis, P. intermedia*, lower education level, irregular dental attendance and increased symptoms of depression were risk factors or risk predictors for attachment loss (15, 24, 36). Although a different longitudinal study of another population was unable to confirm *P. gingivalis* as a risk factor for periodontitis, it did identify the presence of spirochetes as a risk factor for development of periodontitis at previously healthy sites (odds ratio=3.13 to 3.68). Papapanou (92) reviewed longitudinal studies ranging from 2 months to 28 years (16, 17, 24, 50–52, 60) and noted that that tobacco use (smoking and spit tobacco), specific subgingival bacterial species, low education, infrequent dental visits, male sex, lack of flossing, and race (African-American) were statistically significant risk factors or risk predictors for clinical attachment loss. Although race and socioeconomic status are often associated with variations in periodontal disease, these differences have not been observed in studies when periodontal status was adjusted for oral hygiene and smoking (39). It is also interesting to note that depression has been associated with disease activity (24, 36, 87). Very recently, an analysis of over 11,000 women in a large epidemiological study revealed that the presence of severe osteoporosis in the presence of high levels of dental calculus was a risk indicator for clinical attachment loss and gingival recession but not for increased pocket depth (105). Recent longitudinal data have confirmed that osteoporosis in women is a risk factor for loss in alveolar bone density (94).

Genetic factors have also been associated with periodontitis. Independent studies of twins in Minnesota and Virginia both reported that there is a significant genetic component in chronic periodontitis in adults (32, 84, 85). It has been estimated that between 38% and 82% of the population variance for gingivitis, probing depth and clinical attachment loss is due to genetic variation (85). Moreover, a mutation in the region of chromosome 11q14 that contains the cathepsin C gene for prepubertal periodontitis was recently identified (54). Kornman et al. reported that a specific interleukin 1 (IL-1) genotype was associated with severe periodontitis (67). While Gore et al. were unable to confirm this association, they did find that a similar IL-1 genotype was more prevalent in adults with chronic periodontitis (43). In the Kornman et al. study, the odds ratio of having the specific IL-1 genotype in severe versus mild disease in nonsmokers was 6.8, but there was no association of this genotype and disease in smokers (67). This supports the theory that specific environmental factors can be such strong risk factors that they overwhelm any genetically determined susceptibility or resistance to disease.

In summary, confirmed risk factors for periodontitis in adults include genetic influences, smoking, diabetes, race, *P. gingivalis*, *P. intermedia*, low education and infrequent dental attendance. The presence of furcation involvement, tooth mobility and a parafunctional habit without the use of a biteguard are associated with a poorer periodontal prognosis following periodontal therapy. Several other specific periodontal bacteria, herpesviruses, increased age, male sex, depression, race, traumatic occlusion and female osteoporosis in the presence of high levels of dental calculus have been shown to be associated with loss of periodontal support in cross-sectional studies and can be considered to be risk indicators of periodontitis. Although all risk factors cannot be modified, it is now possible to identify people at risk for progressive periodontal disease and intervene to alter or modify some of their risks.

Diagnosis

Diagnosis may be defined as identifying disease from an evaluation of the history, signs and symptoms, laboratory tests, and procedures (6). An accurate diagnosis can only be made by a thorough evaluation of data that have been systematically collected by: l) patient interview, 2) medical consultation as indicated, 3) clinical periodontal examination, 4) radiographic examination and 5) laboratory tests as needed. Excellent reviews of diagnostic techniques in periodontology have been published recently (7, 9, 70, 93). Rather than present an extensive review of all diagnostic methods, the following section is an overview of present and future diagnostic methods that may be useful in general dental practice.

Patient interview

The patient interview includes information concerning the source of referral, chief complaint, symptoms and medical and dental history. The source of referral may be important if another dentist or physician referred the patient and may be a valuable asset in diagnosis. An example would be a referral of a patient with leukemia or human immunodeficiency virus (HIV) infection from a physician. For such a patient, it would be critical that the referring physician be contacted in order to obtain an accurate and thorough medical history.

The chief complaint of the patient should be recorded so it may be used for future reference. However, chronic inflammatory periodontal disease is usually painless, and most patients do not have a periodontitis-related chief complaint. They may have become so accustomed to the symptoms of periodontal disease that they do not notice them. Some people may not even notice teeth becoming loose because their periodontitis has been gradually progressive over a number of years. For the majority of patients with chronic inflammatory periodontal disease, the dentist must detect the disease. One cannot wait for patients to develop symptoms because they often occur only in the late stages of disease. When patients with chronic gingivitis do have a chief complaint, it is most often bleeding during toothbrushing. Patients with chronic periodontitis may complain of tooth migration, development of diastemas between the teeth or periodontal swelling associated with an abscess. The history of the chief complaint consists of more specific information about the periodontal symptoms. For example, a patient with a chief complaint of gingival bleeding during toothbrushing for the past 2 weeks might inform the dentist that the bleeding is not associated with swelling or pain and that it stops soon after brushing. The diagnosis for such a patient would be much different than if the patient had reported that the bleeding continued for several hours after toothbrushing and was associated with generalized swelling of the gingiva. In the first example, one might suspect marginal gingivitis and in the second example, serious blood dyscrasia may be involved.

A current medical history must be taken before starting the clinical examination. The minimum that must be known is whether the patient is under the care of a physician, is taking any medication or has any medical condition that may affect the periodontal diagnosis or treatment. Examples of such conditions include: cardiac disease, heart murmur, rheumatic fever, congenital heart disease, prosthetic heart valve or joint replacement, kidney or liver disease, pregnancy, hypertension, diabetes, allergies, abnormal bleeding, infectious disease, disease of the blood or blood-forming organs, malignancy or previous treatment for malignancy. Any history of tobacco use or substance abuse should also be recorded. It should always be remembered that patients often consider themselves in "good health" when they have very significant medical problems that may influence their dental or periodontal care. It is the dentist's responsibility to obtain an accurate and complete medical history, and this should not be delegated to others.

The history of oral care will determine whether the patient has had previous treatment that affects the diagnosis or treatment plan. For example, a patient with a history of repeated and extensive periodontal therapy may have refractory periodontitis that cannot be effectively treated with conventional therapy. In such cases, the dentist should obtain permission from the patient to request previous dental records. Detailed information about previous periodontal diagnoses and treatment can be extremely helpful in developing a treatment plan for patients with periodontal disease.

Medical considerations and consultation

Medical consultation should be obtained when the medical history indicates a need for more information. A common example is when patients give a history of a heart murmur or joint replacements. Current recommendations preclude periodontal probing or any procedure that may induce bleeding in all patients with high or moderate risk for endocarditis unless antibiotic prophylaxis is provided (33). According to other recommendations, patients with orthopedic pins, plates and screws do not need antibiotic prophylaxis, nor is it routinely needed for most dental patients with total joint replacements (5). However, it is advisable to consider prophylaxis in some patients who may be at increased risk for

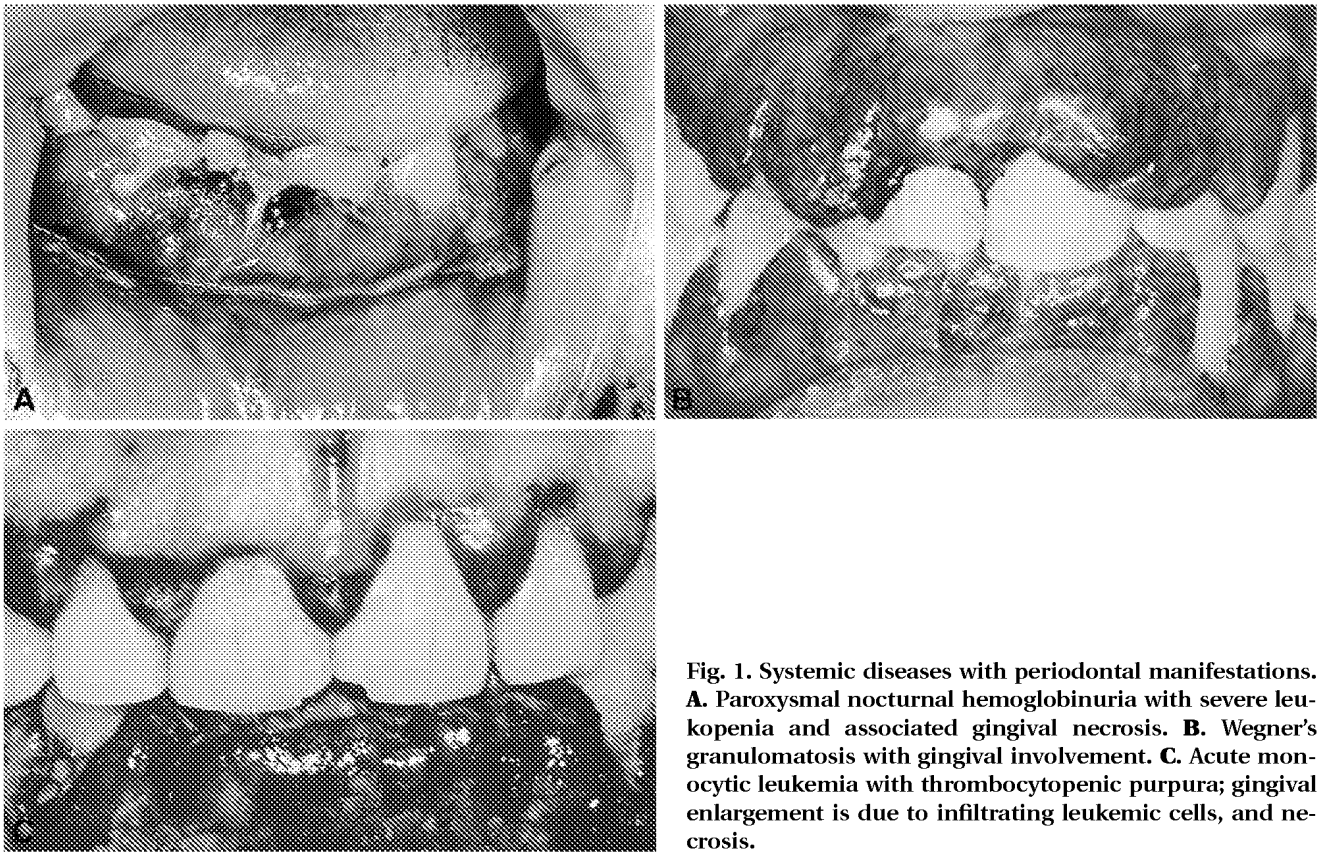

Fig. 1. Systemic diseases with periodontal manifestations. A. Paroxysmal nocturnal hemoglobinuria with severe leukopenia and associated gingival necrosis. **B.** Wegner's granulomatosis with gingival involvement. **C.** Acute monocytic leukemia with thrombocytopenic purpura; gingival enlargement is due to infiltrating leukemic cells, and necrosis.

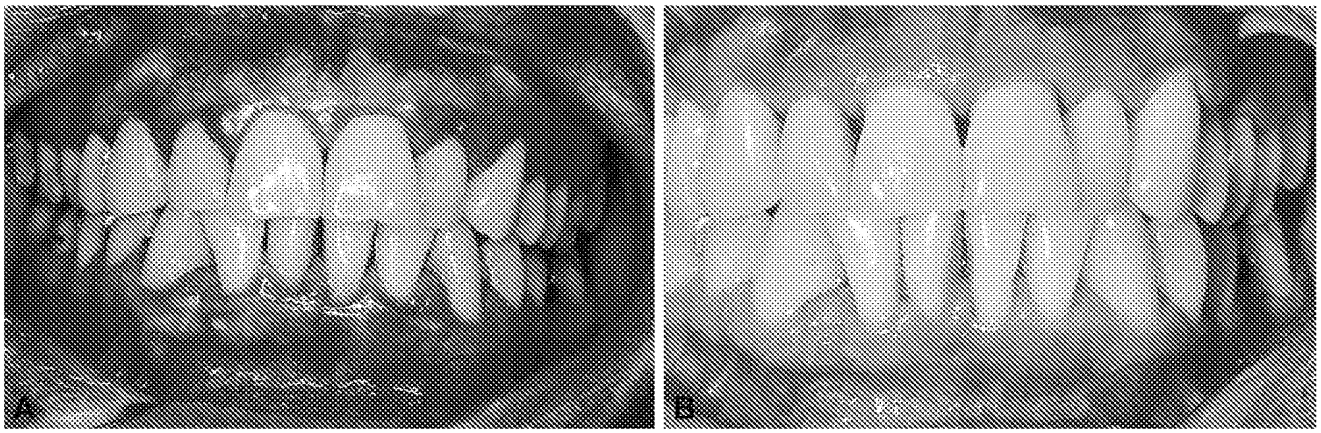

Fig. 2. A. Periodontal inflammation due to plaque and subgingival calculus. B. Same patient after improving oral hygiene and meticulous scaling and root planing to remove all subgingival calculus.

hematogenous joint infection, and it is important to consult with the patient's physician before procedures are done that cause bacteremia in patients who have had total joint replacement.

There are many other findings in the medical history that may require medical consultation such as a history of coronary disease, malignancy, allergy, acquired immunodeficiency syndrome (AIDS), diabetes, blood dyscrasia and endocrine or skin diseases. A review of systemic conditions that may have clinical manifestations in the oral and craniofacial complex is beyond the scope of this chapter. However, some diseases with periodontal manifestations may be life threatening. Prompt and accurate diagnosis followed by effective treatment is essential for such patients. Examples of systemic diseases with periodontal manifestations are given in Fig. 1. Many systemic diseases may influence the diagnosis or treatment plan of a patient with periodontal disease, and it is important that the dentist have all necessary information about the medical status of patients before establishing a diagnosis and treatment plan. A good rule to follow is that, when in doubt, always consult.

Clinical examination

The clinical examination should include an examination of the extra- and intraoral tissues, temporomandibular joints, teeth, occlusion and the periodontium. Any abnormal findings should be recorded and used to develop a definitive diagnosis or further investigated by referral or biopsy. The teeth are examined for caries, prominent wear facets, uneven marginal ridges, open contacts, malposition, failing restorations, evidence of food impaction and tenderness to percussion. Pulp testing is accomplished as indicated by the history or the periodontal and/or radiographic examination. The occlusion is examined for centric, working, non-working and protrusive interferences. Evidence of possible occlusal trauma as indicated by fremitus (mobility in function) is recorded. Fremitus is best determined by having the patient make excursive mandibular movements and close repeatedly in habitual centric occlusion while the dentist feels for tooth movement with an index finger placed lightly on the buccal surfaces of the teeth.

Clinical periodontal examination

The periodontal tissues are routinely examined in all oral examinations. The periodontal examination consists of a visual inspection of the gingiva, periodontal probing, and assessment of tooth mobility, dental plaque and calculus. A specific form should be used for recording dental and periodontal findings so that they may be compared over time. Examinations such as the Periodontal Screening and Recording Examination™ (PSR®) that has been endorsed by the American Academy of Periodontology and the American Dental Association may be useful to the general practitioner (3). However, it is important to recognize that during such screening procedures, all surfaces of the teeth must be probed for signs of periodontal disease.

The gingiva should be visually examined for signs of inflammation. Healthy gingiva, in the absence of significant melanin pigmentation, is normally a light pink color. Increased redness or erythema is a clinical sign of gingival inflammation because of the increased gingival vascularity in response to local irri-

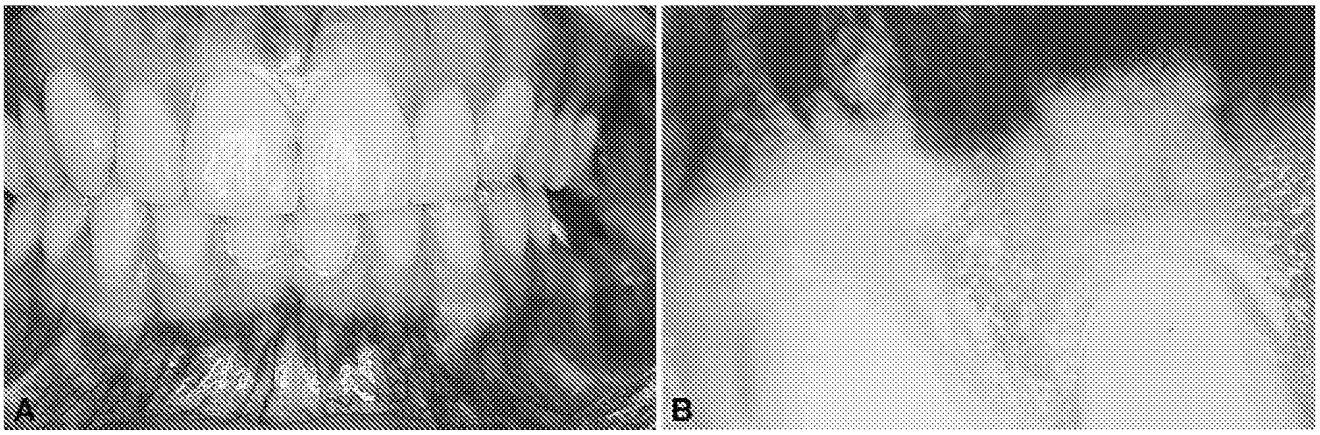

Fig. 3. A. Healthy periodontal tissues with minimal inflammation. B. Close-up of healthy gingiva showing lack of edema and swelling with knife-edged free gingival margin.

tants such as dental plaque and calculus (Fig. 2). The architecture of the gingiva should be examined for changes in the normal knife-edged appearance of the free gingival margin and interdental papilla as it meets the teeth (Fig. 3). In the absence of systemic disease or drug-associated gingival enlargement, any swelling or an enlarged appearance of the marginal gingiva is a sign of inflammation. The consistency of any gingival enlargement should be evaluated with the side of a periodontal probe to determine whether it is edematous or fibrotic. Another clinical sign of gingival inflammation is bleeding from the gingival crevice when the inner aspect of the gingival sulcus is gently swept with the side of a periodontal probe. Any significant lack of attached gingiva, especially if it is associated with gingival recession or a high frenum attachment, should be noted and recorded in the dental record. Evidence of interdental cratering is especially important if it is accompanied by necrosis of the gingiva with or without exposure of the underlying bone. Interdental necrosis (Fig. 4) may be a clinical sign of necrotizing ulcerative gingivitis or necrotizing ulcerative periodontitis. It may also be a clinical sign that is associated with immunocompromised patients with AIDS (40).

Periodontal probing is done on all surfaces of every tooth in the dentition (Fig. 5). During probing,

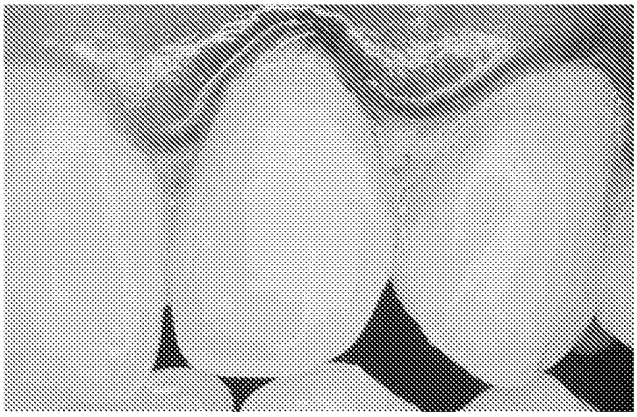

Fig. 4. Interdental necrosis mesial and distal to the lateral incisor that is characteristic of necrotizing ulcerative gingivitis

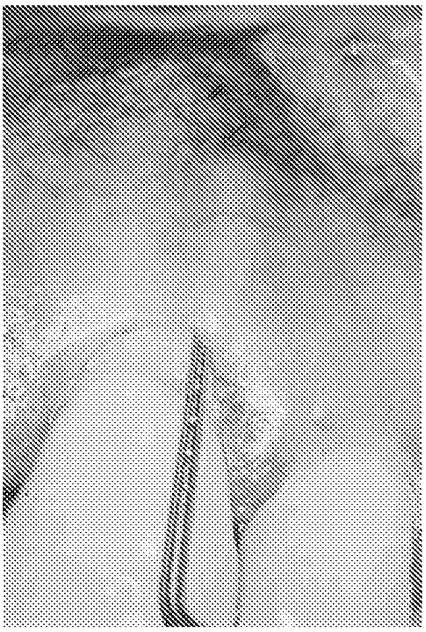

Fig. 5. Periodontal probing: pocket depth=6 mm; clinical attachment level=8 mm. Probing depth=distance from free gingival margin (FGM) to base of pocket or crevice. Clinical attachment loss=distance from cementoenamel junction (CEJ) to base of pocket or crevice. When there is no gingival recession, clinical attachment loss=pocket or crevice depth minus distance from the cementoenamel junction to the free gingival margin.

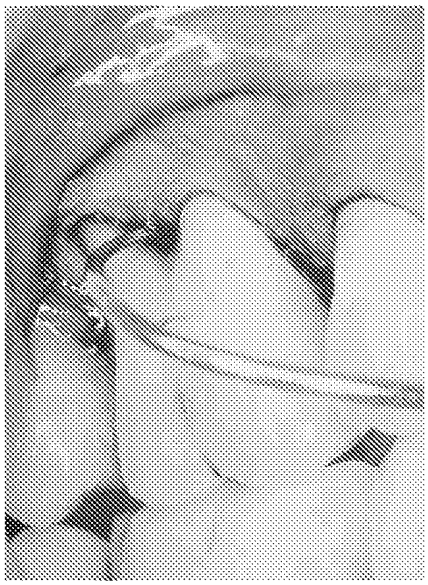

Fig. 6. Class IV furcation involvement

a thin periodontal probe should be used with gentle pressure and it should be "walked" around the entire circumference of each tooth. Probing depth and clinical attachment level should be recorded for all teeth at each of six locations (buccal, lingual, mesiobuccal, mesiolingual, distolingual and distobuccal). Probing depths greater than 3 mm and clinical attachment level greater than 1 mm should be recorded on an appropriate form. Clinical attachment loss is the distance from the cementoenamel junction to the apical extent of the pocket and represents the best clinical measure of disease severity in terms of loss of support for the teeth. Recording clinical attachment level allows one to monitor stability of periodontal health or document disease progression over time. It is important to document furcation involvement because teeth with periodontal pockets in furcations have been shown to have increased loss of attachment and a poorer prognosis following periodontal therapy than teeth without furcation involvement (81, 116). Furcations can be probed with an explorer to determine extension of pockets into areas between roots (Fig. 6). The extension of pockets into furcations is recorded using a classification such as that given below (26, 41).

- I=incipient or early suprabony pocket extension into the furcation area with slight loss of bone;
- II=extension of the pocket into the furcation leaving a portion of the alveolar bone and periodontal ligament intact allowing only partial penetration of the probe into the furcation area;
- III=through and through extension of the pocket into the furcation with complete loss of inter-radicular bone without gingival recession; and
- IV=through and through furcation invasion with gingival recession (Fig. 6).

Gingival recession (Fig. 7) is also recorded during periodontal probing as the distance of the free gingival margin to the cementoenamel junction. In addition to the amount of recession, the morphology of specific areas of gingival recession should be recorded in terms of width and its relation to the interdental papilla. The following is a clinically useful and widely accepted classification (86):

- Class I=recession that does not extend to the mucogingival junction and is not associated with loss of bone or gingival tissue in the interdental area;

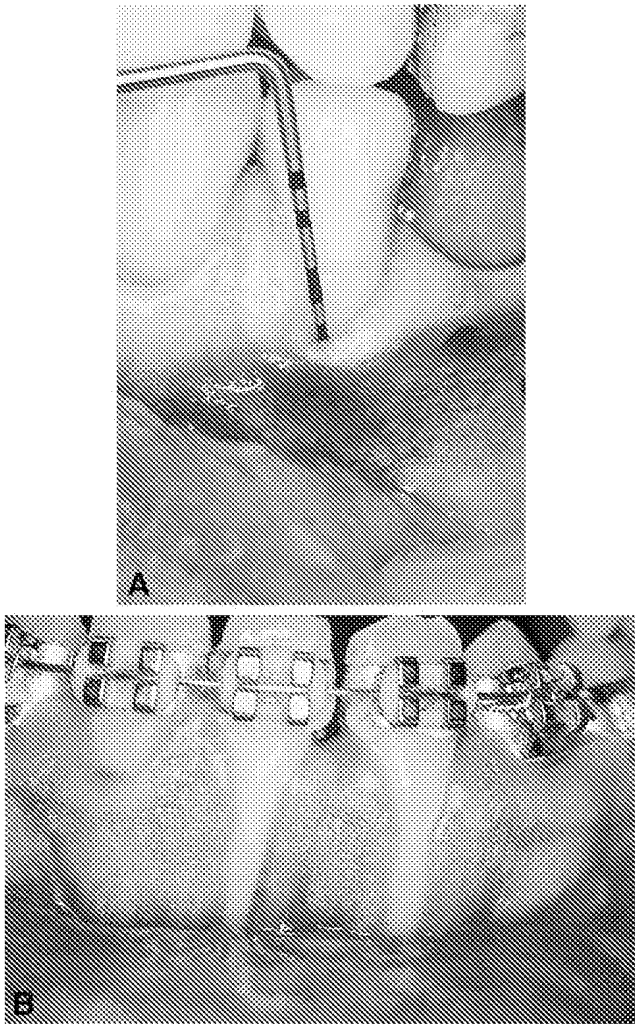

Fig. 7. A. Miller class III gingival recession measured with color-coded periodontal probe. B. Miller class II gingival recession.

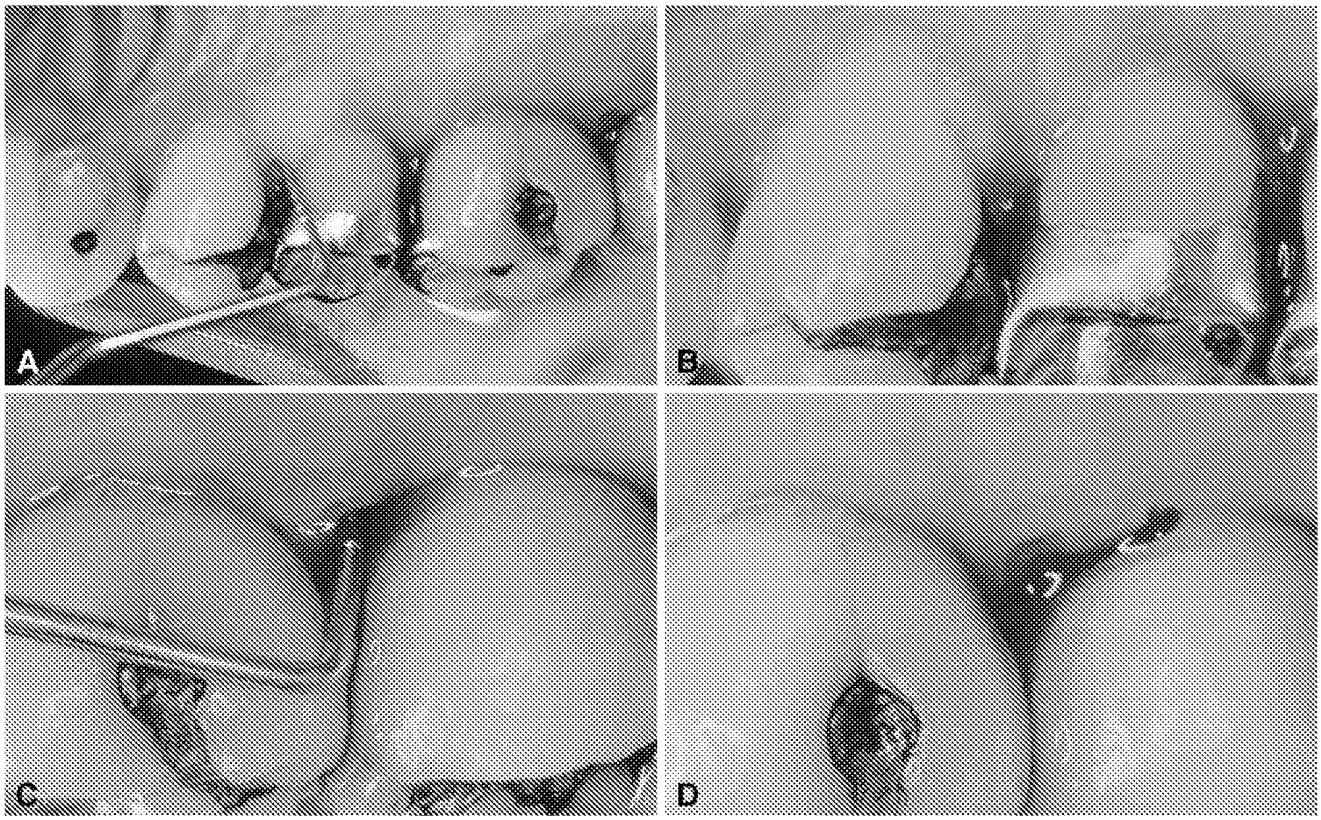

Fig. 8. Bleeding and suppuration after probing. A. Probing depth on disto-lingual surface of maxillary first bicuspid= 5 mm, attachment loss=5 mm. B. Suppuration after probing disto-lingual surface of first premolar. C. Probing depth on mesiolingual of first molar=9 mm, attachment loss=10 mm. D. Bleeding after probing mesiolingual surface of first molar.

- Class II=recession that extends to the mucogingival junction and is not associated with loss of bone or soft tissue in the interdental area;
- Class III=recession that extends to or beyond the mucogingival junction with loss of bone or soft tissue in the interdental area; and
- Class IV=recession extending to or beyond the mucogingival junction with severe loss of interdental bone and/or soft tissue and/or severe tooth malposition.

Bleeding or suppuration on probing is recorded (Fig. 8). Many practitioners find that using a Bleeding Index to document the percentage of sites that bleed on probing is helpful in monitoring the progress of therapy. For example, if bleeding on probing is found at 75 of 168 possible sites in a patient with 28 teeth, a bleeding index of 45% would be recorded (75/168×100=45%). Subsequent measures of the bleeding index can give an objective measure of the effectiveness of therapy over time in reducing periodontal inflammation.

Many types of periodontal probes are currently available. In general, these can be divided into first-, second- and third-generation instruments (96). First-generation probes include conventional periodontal probes, second-generation probes utilize controlled forces and third-generation probes incorporate automated measurement, controlled forces and computerized data capture. First-generation color-coded or banded probes such as the Williams, University of North Carolina or the University of Michigan probes (Hu-Friedy, Chicago IL) are simple and easy to use and offer excellent tactile sensitivity. Second-generation probes such as the Brockprobe™ (Brockport Industries, Hackettstown NJ), PDT Sensor Probe™ (Batesville, AK) and the TPS Probe® (Vivacare, Schaan, Liechtenstein) permit the use of constant pressure during probing. Third-generation probes such as the Florida Probe® (Florida Probe Corp., Gainesville, FL) and the Probe One® (American Dental Technologies, Corpus Christie, TX) have the advantages of direct data entry using computer software and controlled force. Although available third-generation probes do not currently measure clinical attachment level, the Florida Disc Probe®

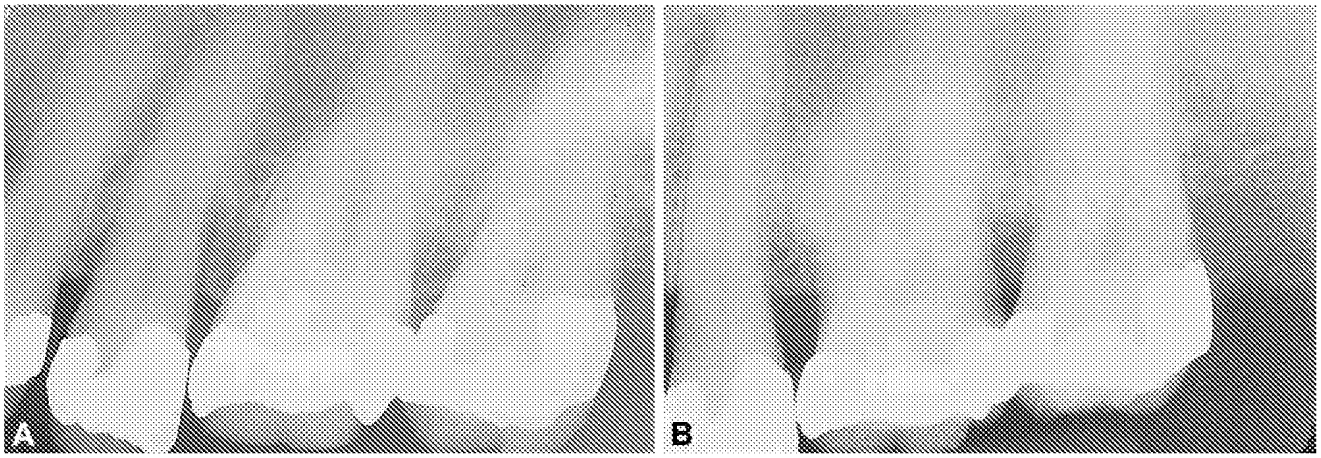

Fig. 9. Radiographs obtained of the same tooth using a bisecting angle method (A) and a parallel long-cone method (B). Note distortion of interdental bone on bisecting angle radiograph (A) so that crest is projected coronal to the cementoenamel junction. Note overlapping of buccal and lingual cusps on parallel technique film (B) and realistic projection of interdental crest between molars.

(Florida Probe Corp., Gainesville, FL) allows relative attachment level to be measured using the occlusal surfaces of the teeth rather than the cementoenamel junction as a reference landmark.

Tooth mobility should be recorded because teeth that are mobile have been shown to have a poorer prognosis and increased attachment loss after periodontal therapy (81, 116). Mobility is recorded by moving the teeth in a buccolingual and occlusoapical direction. Slight mobility, beyond that which is physiological, is given a score of 1. If the mobility is somewhat more but the tooth cannot be depressed apically in the alveolus, it is scored a 2. If the mobility is advanced to the degree that the tooth may be depressed apically, it is graded as 3 (26). An electronic instrument (Periotest®, Siemens AG, Mannheim, Germany) is commercially available for measuring tooth mobility and may be useful for documenting progressive tooth mobility over time.

The presence and distribution of dental plaque and calculus should be recorded. A convenient and useful method of recording plaque is the O'Leary Plaque Control Record (90). It documents the overall percentage and specific location of tooth surfaces with plaque. Briefly, each tooth surface (six surfaces per tooth) is scored for the presence or absence of plaque in contact with the free gingival margin. The total score is then calculated as the percentage of tooth surfaces with dental plaque. For example, a person having 45 out of a possible 156 tooth surfaces with plaque (26 teeth) would have a plaque score of $45/156 \times 100 = 29\%$. This is a quick and useful method for assessing patient plaque control and for monitoring the effectiveness of patient plaque control programs.

Radiographic examination

Radiographs are used to confirm and extend the findings of the clinical examination and are essential in planning implant placement to determine the amount and character of alveolar bone as well as the position of anatomical structures such as the maxillary sinus and inferior alveolar canal. The presence of gingivitis, periodontal pockets and gingival inflammation cannot be determined using radiographs, but radiographs are essential for determining the extent and severity of bone periodontal support and for detecting osseous lesions. Although panoramic radiographs provide an excellent radiographic survey of the oral structures, they lack the resolution and detail needed for periodontal diagnosis. When the clinical examination indicates the presence of periodontitis, selected periapical or bitewing radiographs should be obtained (4). A full mouth intraoral radiographic examination is appropriate when patients have clinical evidence of generalized dental disease or a history of extensive dental treatment (4). Periapical radiographs should be exposed using a long-cone paralleling technique because the bisecting angle technique distorts the relationship between the alveolar crest and tooth (Fig. 9). There are several commercially available devices that facilitate radiographic positioning and long cone projection using a paralleling technique. More-

over, use of E speed film and rectangular collimation should be used because it reduces radiographic exposure by a factor of 8 compared to circular collimation and D speed film (38).

When there is no loss of osseous support, the interdental septum is parallel to a line projected between the adjacent cementoenamel junctions and located slightly apically (about 1 to 2 millimeters) to this imaginary line (19, 103, 108). Periapical radiographs may also give an indication of thickening of the periodontal ligament space on the mesial and distal surfaces that may be associated with traumatic occlusion. While interproximal craters are usually not visible on radiographs, infrabony deformities may be visualized depending on the morphology of bone loss. Hemi-septa or one-walled proximal infrabony defects usually may be easily identified on radiographs, and defects with multiple walls may also be visualized (Fig. 10). Loss of bone in furcations is not readily identified unless there is extensive bone loss or through and through furcation involvement (class III or IV) with loss of bone on both sides of the furcation and between the roots (Fig. 11). While radiographs assist the clinician in determining the degree of osseous support, they underestimate the severity of actual bone loss (114).

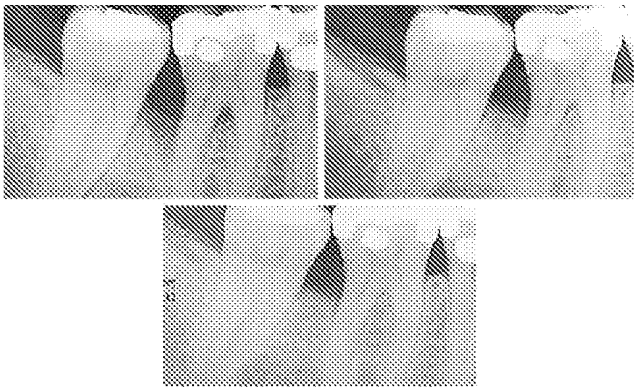

Fig. 10. Upper left. Multiple walled defect on distal surface of mandibular second molar. **Upper right.** Eight months following thorough scaling and root planing. **Bottom.** Sixteen months following scaling and root planing showing apparent regeneration of bone.

Fig. 11. Radiograph demonstrating advanced loss of bone in furcation areas of mandibular molars

Bitewing radiographs are useful for monitoring proximal osseous support of the posterior teeth. If taken at a right angle to the long axis of the teeth without horizontal angular distortion, vertical bitewing radiographs are useful for monitoring both caries and crestal bone height during recall or supportive periodontal therapy. They provide a relatively non-distorted image of the interdental bone of both maxillary and mandibular teeth on the same film.

Digital radiography

Digital radiography has many advantages compared to conventional radiography and is becoming more widely used in dentistry (61). The advantages of digital radiography include immediate image acquisition without the need for film processing, the ability to adjust images to improve diagnostic utility and the ability to electronically store or print images. It also allows the comparison of sequential images over time using subtraction radiography (55). Decreased radiation may be an advantage of digital radiography but only if used with a special computer controlled timer. Digital radiography may actually generate more radiation exposure than standard radiography if it is used with a conventional timer (63). Digital radiography allows the detection of as little as 0.54 mm of change at specific periodontal sites and an average full-mouth change of 0.1 mm from one visit to the next (57). Because of their many advantages, it is likely that digital radiography and computerized image processing will continue to gain acceptance as diagnostic aids in periodontics.

Medical laboratory tests

Medical laboratory tests are indicated when more information is needed about the patient's medical status or to help the dentist more precisely determine the cause or prognosis of periodontal disease. An example would be when a patient is taking anticoagulant medication such as warfarin sodium (Coumadin®). For such a patient, it would be essential for the dentist to know the appropriate laboratory coagu-

lation values such as the prothrombin time or INR. Laboratory tests that are ordered to clarify the patient's medical status are best done in consultation with the patient's physician because it facilitates appropriate medical management during periodontal therapy.

Disease activity and periodontal diagnostic tests

The utility of diagnostic tests is defined in terms of statistical sensitivity, specificity, positive predictive value and negative predictive value (Table 2). The statistical sensitivity of a diagnostic test is defined as its true positive rate. In other words, it is the probability that a test is positive if the disease is present. The statistical specificity of a diagnostic test is its true negative rate, or the probability that a test is negative when the disease is absent. The positive predictive value is the probability that the disease is present when the test is positive, and the negative predictive value is the probability that the disease is absent when the test is negative. Although any diagnostic test would ideally have a value of 1.0 for all of these values, no test is perfect in this regard. In general, the sensitivity and specificity for any diagnostic test should be at least 0.7 (9). However, the acceptable value for any of these statistical parameters depends on the consequences of a missed diagnosis as well as the risks and morbidity associated with treatment. For a disease that is invariably fatal, but is easily treated with minimal morbidity and risk, one would want to maximize test sensitivity. For such a disease, the consequences of not diagnosing the disease are catastrophic especially since treatment is effective, safe and has few side effects. The specificity and negative predictive value of the diagnostic test in this example are less important because the consequences of treating the disease in its absence are not severe. Conversely, if treatment for a disease has serious side effects or high risk, one would want a test with a high specificity (true negative rate) so that one does not receive treatment in the absence of disease.

The predictive values of any test are influenced by disease prevalence in the population. As disease prevalence increases, the positive predictive value of a test increases and the negative predictive value decreases. With low disease prevalence, the positive predictive value decreases and the negative predictive values increases. Moreover, disease prevalence in any population is dependent on the "gold standard" diagnostic test that is used to define the true presence or absence of disease (98).

Table 2. Test sensitivity=A/A+C; specificity=D/B+D; positive predictive value=A/A+B; negative predictive value=D/C+D

	Disease present	Disease absent	
Positive test	A (True +)	B (False +)	A+B
Negative test	C (False −)	D (True −)	C+D
	A+C	B+D	

Clinical signs of periodontal disease and disease activity

Clinical signs of periodontal disease such as pocket depth, loss of clinical attachment and bone loss are cumulative measures of past disease. They do not provide the dentist with an assessment of current disease activity. Indeed, current probing depth is not very predictive of future disease progression in the near term (5–12 months) since the positive predictive value for future attachment loss of pockets >4 mm is in the range of 0.02–0.45. Furthermore, other clinical findings such as redness (positive predictive value=0.02–0.05) and the presence or suppuration (positive predictive value=0.02–0.82), while indicative of ongoing inflammation, do not appear to be very useful in terms of predicting future attachment loss (7). It should be noted that the higher positive predictive values for suppuration or probing depth only occur in populations that have a high incidence of disease progression (7). As noted by Armitage, suppuration has a stronger association with disease progression than redness, but its relative rarity and lack of standard detection methods make it a poor candidate for predicting progressive periodontitis (7). Based on clinical experience, the presence of pus is viewed as unfavorable, and its high negative predictive value (0.85–0.98) supports this opinion (7). However, data also support that the suppuration is not a good stand-alone predictor of disease progression (7).

Bleeding on probing has also been shown to have a low positive predictive value (range=0.01–0.41) and a high negative predictive value (range=0.86–0.98). In other words, bleeding on probing does not appear to predict future disease progression at individual sites very well, but the absence of bleeding on probing is an excellent predictor of stability. However, as noted by Armitage (7), there is evidence that patients with many areas of bleeding on probing, deep pockets and advanced loss of clinical attachment are more likely to experience future attachment loss (30, 50). Moreover, based on a meta-analy-

sis of three studies involving treated and maintained patients (64, 71, 72), subjects with a high frequency of bleeding (≥50%) at recall visits during a 1-year period were 2.79 times (odds ratio) more likely to develop attachment loss (7). Overall, however, although common clinical findings in inflammatory periodontal disease are critical for establishing a diagnosis, they do not appear to be strong indicators of future disease progression at specific sites. In an attempt to better predict future disease progression, several types of diagnostic tests for the periodontal diseases have been studied. Tests that could accurately predict future disease progression would allow clinicians to better monitor the results of periodontal therapy and prevent recurrent periodontal destruction.

Biochemical assays of gingival crevicular fluid

Inflammation is associated with vascular exudation and this serum exudate can be collected from the gingival crevicular fluid (Fig. 12) and analyzed to assess the inflammatory process in biochemical terms. These biochemical gingival crevicular fluid assays have been studied in an attempt to predict disease progression before it becomes evident using routine clinical or radiographic assessment. While such assays are not routinely used in clinical practice today, they may prove to be valuable indicators of active disease in the future. In general, these assays may be classified into three general groups: 1) products and mediators of inflammation, 2) host-derived enzymes and 3) tissue breakdown products (7).

Host inflammatory products and mediators of inflammation that have been studied as possible diagnostic markers of periodontal disease include prostaglandin E_2, cytokines, antibacterial antibodies, total protein and acute phase proteins (7). Among these, prostaglandin E_2, the interleukins (interleukin-1β, interleukin-6 and interleukin-8) and tumor necrosis factor-α have received most attention as potential candidates for markers of disease progression. The pro-inflammatory cytokines interleukin-1β and tumor necrosis factor-α have a broad range of effects in tissue and have been associated with bone resorption. Indeed, there is evidence in a primate animal model that progressive inflammation and osteoclast formation in experimental periodontitis is inhibited by local application of interleukin-1 and tumor necrosis factor–blocking agents (44). Many studies have established that these and other cytokines are either increased or decreased in periodontal disease on a cross-sectional basis (7, 68, 80, 91). There is also recent evidence that a reduction in gingival crevicular fluid interleukin-1β following periodontal treatment is linked to patient genotype (37). However, additional longitudinal evidence is needed before these cytokines can be employed in routine general dental practice as predictors of future disease progression. Moreover, although total protein, antibacterial antibodies and acute-phase proteins have been studied in terms of their association with periodontal disease, none has emerged for use as a marker of active periodontal disease.

Host-derived enzymes such as aspartate aminotransferase, neutral protease, collagenase, β-glucuronidase, lactate dehydrogenase, neutrophil elastase, arylsulfatase, myeloperoxidase and alkaline phosphatase have been investigated for their association with periodontal disease and as markers of periodontal inflammation (7, 68, 69, 92). Several of these have been shown to be elevated in the gingival crevicular fluid of failing implants compared to successful implants and may be good candidates for risk markers of implant failure (22). Enzyme tests for aspartate aminotransferase (PocketWatch™, Steri-Oss, Yorba Linda, CA) and neutral proteases (Periocheck®, CollaGenex Pharmaceuticals, Newtown, PA) are available as chairside gingival crevicular fluid tests. Aspartate aminotransferase has been shown on a cross-sectional and longitudinal basis to be associated with periodontal inflammation and loss of attachment (28, 59, 78, 95). Neutral protease has been reported to be elevated in patients with periodontal disease (23) and has been reported to have a high sensitivity (88%) as a diagnostic test for clinical

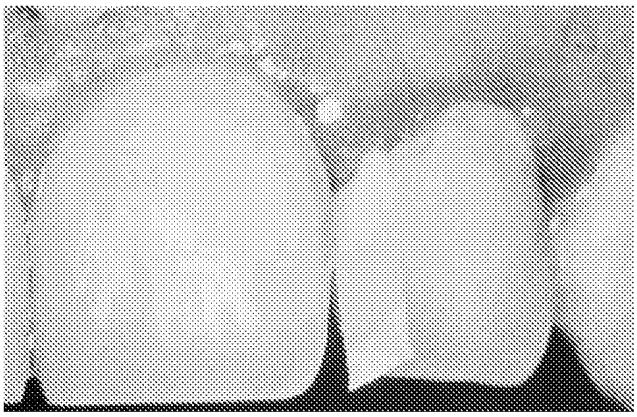

Fig. 12. Collection of gingival crevicular fluid from inflamed gingiva using an absorbent strip of paper (Periopaper®, OraFlow, Plainview, NY)

disease (56). However, there is conflicting evidence regarding the utility of this enzyme for predicting disease progression (11).

Tissue breakdown products such as glycosaminoglycans, hydroxyproline, fibronectin, connective tissue proteins and calprotectin have been related to clinical measures of disease (7, 65) and may prove to be helpful in the future for identifying sites or patients that are undergoing progressive destruction. In his review of gingival crevicular fluid diagnostic tests, Lamster noted that while many gingival crevicular fluid tests hold promise for the future as diagnostic tests, it is not known if test results in patients with gingivitis can be used to predict future periodontitis (68). Furthermore, he noted that the validity of gingival crevicular fluid–based diagnostic tests needs to be established in terms of sensitivity, specificity, and predictive values for future disease (Table 2). It is also unclear whether the results of such tests are applicable to individual sites or if they are best applied to the patient in terms of predictive value. In other words, full-mouth averages of gingival crevicular fluid enzymes may be more useful for predicting patients at risk than the use of gingival crevicular fluid enzymes for predicting specific sites at risk for disease progression.

Subgingival temperature

Subgingival temperature has been proposed as a diagnostic aid. A commercially available device (Periotemp®, Abiomed, Danvers, MA) that resembles a periodontal probe is used to measure subgingival temperature to a precision of 0.1°C. Subgingival temperature is increased in gingival inflammation (48), and there is evidence that increased mean subgingival temperature is related to increased risk for clinical attachment loss (49). However, since a major influence on subgingival temperature at individual sites is the anatomic location within the mouth (79), temperature variation at various sites in the mouth may not be as useful as overall differences in mean subgingival temperature for predicting future disease activity.

Microbiological testing

Although microbiological testing is not indicated for the majority of periodontal patients, it may help the dentist to more precisely define the cause of periodontal disease and guide therapy for specific patients. For example, microbiological testing may be indicated for patients with juvenile, refractory or rapidly progressing periodontitis. Patients with juvenile periodontitis may have large numbers of *Actinobacillus actinomycetemcomitans* (121). Adults with refractory periodontitis may harbor large numbers of *B. forsythus, P. gingivalis, P. intermedia, Campylobacter rectus, Eikenella corrodens, Eubacterium* species, *Peptostreptococcus micros, Selenomonas* species and spirochetes (121). It is important to know whether such patients have persistent periodontal infections with these organisms and to know whether the organisms are sensitive to specific antibiotics. This allows the dentist to control or treat disease by combining mechanical debridement with appropriate antimicrobial chemotherapy.

There are a variety of methods of assessing the bacterial flora of patients with periodontal disease. Typically, plaque samples are collected with a curette or a paper point (Fig. 13). These samples can be analyzed using phase-contrast or dark-field microscopy, bacterial enzyme analysis, immunoassay, DNA probes, polymerase chain reaction or traditional microbiological culturing and sensitivity. Microscopy has been used for assessing motile organisms and spirochetes during treatment and maintenance therapy (73, 120). However, individual bacterial species cannot be identified with routine phase or darkfield

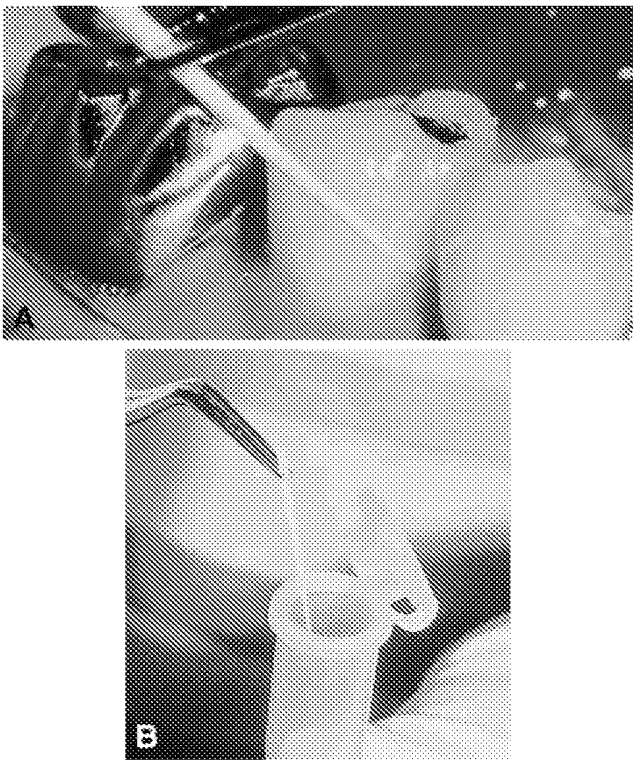

Fig. 13. A. Collection subgingival plaque using sterile paper point. B. Insertion into a vial for transport to the laboratory for microbial analysis.

microscopy, and it may not provide sufficient benefits to justify the additional time and labor that is required for its use in preventive periodontal maintenance (73). Some periodontal bacteria (*B. forsythus, P. gingivalis* and *Treponema denticola*) produce enzymes that are capable of hydrolyzing a synthetic peptide (BANA), and this has been used in a chairside detection test (Perioscan®, Oral-B Laboratories, Belmont, CA) (74, 75). Other assays utilize antibodies or DNA probes of nucleic acid sequences to identify specific bacterial species. These tests are very sensitive and can detect as few as 100 to 1000 bacteria. Polymerase chain reaction technology is the most sensitive test currently available for detection of viruses and bacteria. Polymerase chain reaction methods amplify exceedingly small amounts of bacterial nucleic acid and can detect as few as 10 organisms in a plaque sample (115). Although these tests provide useful information, the only bacterial assay that can determine whether bacteria are sensitive to specific antibiotics is laboratory culturing and sensitivity testing. As noted by Armitage (7) in his review of microbiological testing, potentially useful information that might be gained from such testing includes the identification of putative periodontal pathogens or unusual superinfecting organisms and antibiotic sensitivity. It is important to use appropriately licensed microbiological laboratories that routinely perform culture and sensitivity tests for periodontal bacteria, because most medical laboratories do not routinely screen for these microorganisms. It must be emphasized that the vast majority of periodontal patients do not require microbiological testing for diagnosis or to provide effective therapy. Microbial analysis should be reserved for patients who have unusual forms of periodontal disease such as early-onset, refractory or rapidly progressive disease.

Genetic tests

Recently, a genetic test (PST™, Medical Science Systems, San Antonio, TX) has become available to test patients for periodontal disease risk (67). This test determines if people possess a combination of alleles in two interleukin-1 genes. This particular combination has been associated with severe disease in nonsmoking Caucasians. Others (43) reported an increased frequency of a different interleukin-1 genotype in people with advanced adult periodontitis compared to those with early or moderate disease. There is also retrospective evidence that genetic testing for the specific interleukin-1 genotype (PST™) may give an indication of increased susceptibility to tooth loss in periodontal maintenance patients (82). However, a more recent prospective study reported that this same composite genotype was not associated with progressive clinical attachment loss during a 2-year period after periodontal therapy (35). Overall, it may be concluded that genetic testing has potential for future use but that more research is needed to evaluate the utility of various tests for determining genetic susceptibility to the periodontal diseases.

Types and characteristics of periodontal diseases

In general, the periodontal diseases vary in terms of age of onset, causation, clinical characteristics and methods of treatment. Summaries of diseases and disorders that affect the periodontal tissues are given in Tables 3 and 4. For administrative and third-party insurance reporting purposes, the American Academy of Periodontology classifies gingivitis and periodontitis into five broad case types (2). Plaque-associated gingivitis is designated as Case Type I and chronic periodontitis is divided into four case types based on increasing disease severity (II–IV) and lack of response to conventional therapy (Case Type V). Case Type II (early periodontitis) is characterized by progression of inflammation into the deeper periodontal structures with slight bone and attachment loss. Case Type III (moderate periodontitis) is classified as a more advanced state with increased destruction of the periodontal structures and noticeable loss of bone support, possibly accompanied by increased tooth mobility and furcation involvement on multirooted teeth. Case Type IV (advanced periodontitis) is characterized by further progression of periodontitis with major loss of alveolar bone support that is usually accompanied by an increase in tooth mobility. Furcation involvement is a common finding. Case Type V (refractory periodontitis) includes those patients that continue to demonstrate attachment loss after good conventional therapy. These sites presumably continue to be infected by periodontal pathogens regardless of thoroughness or frequency of treatment.

In 1999, the American Academy of Periodontology revised its biological classification of the periodontal diseases (8). This revision included the addition of a section on gingival diseases, replacement of the term "adult periodontitis" with the term "chronic periodontitis", replacement of the term "early-onset periodontitis" with the term "aggressive periodontitis" and elimination of a separate disease cat-

Table 3. Gingival diseases

Disease	Primary age of onset	Primary causes	Signs and symptoms	Therapy
Gingivitis (American Academy of Peridontology case type I)	Any age	Bacterial plaque, local plaque retention factors (such as faulty restorations)	Gingival redness and swelling, bleeding; does not cause loss of clinical attachment	Debridement, plaque control, correct plaque-retentive factors, supportive periodontal therapy
Acute necrotizing gingivitis	Young adults, older children, young malnourished children	Bacterial plaque, may be associated with AIDS at any age	Pain, gingival redness, swelling, bleeding, necrosis of interproximal papilla	Debridement, gentle plaque control, antimicrobial rinse, supportive periodontal therapy
Desquamative gingival disease	Adults	Skin disease: lichen planus, pemphigus and cicatricial pemphigoid	Gingival redness; epithelial denudation; pain with trauma or on eating and brushing	Gentle plaque control, palliative and symptomatic therapy, supportive periodontal therapy
Gingivitis associated with systemic diseases (such as blood dyscrasias and Wegner's granulomatosis)	Any age	Manifestation of systemic disease in gingiva (such as leukemia, neutropenia, erythema multiforme, lupus erythematosis and Wegner's granulomatosis)	Dependent on systemic disease (such as gingival bleeding ecchymosis, redness, swelling, necrosis and pain)	Treatment of systemic disease, atraumatic plaque control, antimicrobial rinse, supportive periodontal therapy
Gingivitis associated with pregnancy	Young females	Bacterial plaque, local plaque retention factors, hormonal influence	Gingival redness and swelling, bleeding; pyogenic granuloma	Debridement and plaque control, supportive periodontal therapy; possible excision of pyogenic granuloma
Drug-induced gingival enlargement	Any age	Ca^{++} channel-blocking drugs, phenytoin, cyclosporine	Gingival enlargement	Debridement and plaque control, surgical excision, use of alternative medications, supportive periodontal therapy
Allergic reaction	May occur at any age; is generally uncommon	Local allergens (such as mouthrinses, toothpastes, nickel restorations and acrylic)	Gingival redness and swelling	Identification and elimination of allergenic agent
Herpetic gingivostomatitis	Primarily children and young adults	Herpes type I virus	Pain, vesicle formation, ulceration	Palliative and symptomatic therapy, antiviral medication
Gingival disease of specific bacteria or fungal origin	May occur at any age but is rare	*Neisseria gonorrhoea*, *Treponema pallidum*, streptococcal species, *Candida*, histoplasmosis	Varies according to infectious agent	Identification and elimination or control of infectious agent, appropriate chemotherapy

egory for "refractory periodontitis". It also included a clarification of the designation "periodontitis as a manifestation of systemic diseases", replacement of "necrotizing ulcerative periodontitis" with "necrotizing periodontal diseases" and additions of disease categories on periodontal abscesses, periodontitis associated with endodontic lesions, and developmental or acquired deformities and conditions. The classification given in Tables 3 and 4 incorporates most of these changes, but retains other disease classifications, such as refractory and juvenile periodontitis, because they are clinical diagnoses that have treatment implications for patients and clinicians. Note that periodontitis associated with endodontic lesions and developmental and acquired deformities are not included in Table 4.

Assessment of data and diagnosis of the periodontal diseases

Traditionally, periodontal diagnosis has been based almost entirely on clinical findings (9). The clinician must rely on: 1) the severity and extent of inflammation, 2) severity and pattern of periodontal pockets and clinical attachment loss, 3) patient age at onset, 4) rate of progression, and 5) miscellaneous

Table 4. Types of periodontitis

Disease	Primary age of onset	Primary causes	Signs and symptoms	Therapy
Chronic periodontitis (American Academy of Periodontology case types II, II, and IV)	Any age	Bacterial plaque, smoking, local plaque retentive factors such as dental calculus and faulty restorations	Overall slow progression with generalized periodontal pockets, bone and clinical attachment loss, may be generalized or localized	Plaque control, smoking cessation, scaling and root planing, correction of local plaque retentive factors, antimicrobial chemotherapy, periodontal surgery, supportive periodontal therapy
Aggressive periodontitis (diseases formerly classified as juvenile periodontitis by the Amercian Academy of Periodontology may also be in this category)	Any age	Bacterial plaque, superinfection with specific periodontal bacteria, possible impaired host response, smoking	Severe and rapid periodontal destruction possibly followed by periods of remission; may be generalized or localized	Specific antimicrobial therapy based on microbial analysis, smoking cessation, debridement, possible periodontal surgery, supportive periodontal therapy
Refractory periodontitis of any type (American Academy of Periodontology case type V)	Any age	Bacterial plaque, superinfection with specific periodontal bacteria, possible impaired host response, smoking	Progression of disease despite good conventional therapy and supportive periodontal therapy	Specific antimicrobial therapy based on microbial analysis, smoking cessation, debridement, possible periodontal surgery, supportive periodontal therapy
Periodontitis as a manifestation of systemic diseases	Any age	Associated with disorders of the blood or blood-forming organs such as neutropenia, leukemia or genetic disorders	Generalized and localized forms of severe destruction of bone and connective tissue tooth support	Treatment of systemic disease, atraumatic plaque control, antimicrobial rinse, supportive periodontal therapy
Juvenile periodontitis: localized and generalized	Near or during puberty	Probably major autosomal gene effect and infection with *Actinobacillus actinomycetemcomitans*	Localizing juvenile periodontitis: typically loss of support in first molar and incisors; generalizing juvenile periodontitis: generalized loss of support throughout dentition	Scaling and root planing, specific antimicrobial therapy based on microbial analysis, possible regenerative surgery, supportive periodontal therapy
Periodontitis associated with endodontic lesions	Any age	May be of endodontic or periodontal origin	Periodontal pocket extending to area of endodontic lesion	If primarily of endodontic origin, endodontic therapy alone; if primarily of periodontal origin, endodontic and periodontal therapy or extraction may be necessary
Periodontal abscess	Any age	Subgingival bacteria	Painful, acute swelling of periodontal tissues associated with deep periodontal pocket	Nonsurgical or surgical debridement, antibiotic therapy; regeneration of lost periodontal support is often a possibility
Acute necrotizing periodontitis	Any age	Immunocompromised, may be associated with HIV	Pain, rapid loss of bone and tooth support associated with gingival and bony necrosis	Debridement, atraumatic plaque control, analgesic medication, antimicrobial rinse, supportive periodontal therapy

signs and symptoms such as pain, ulceration, and amounts of local irritants such as dental plaque and dental calculus. Data from the patient interview, clinical and radiographic examination, and any laboratory tests or medical consultations are thoughtfully assessed to determine an accurate diagnosis. The extent, location and severity of periodontal disease are described in the diagnosis. Any systemic diseases or considerations should also be identified in the diagnosis.

Treatment planning for gingivitis and periodontitis

Gingivitis is a reversible disease and therapy is aimed primarily at eliminating or reducing causative factors. This allows inflammation to resolve and the gingival tissues to heal (99). Treatment for periodontitis generally falls into two categories: 1) procedures designed to halt the progression of disease and 2) procedures designed to regenerate structures destroyed by disease (99). Maintenance or supportive periodontal therapy following active treatment is essential to achieve a successful outcome (102). Many years ago, Ramfjord proposed an overall plan for the treatment of the periodontal diseases (101). This plan included four phases: 1) systemic, 2) hygienic, 3) corrective and 4) maintenance or supportive care. Although the specific details of each treatment phase need updating in light of new information, the basic outline of therapy that he proposed remains valid. It is critical that the diagnosis and treatment plan be presented to the patient in understandable terms. Patients should be informed of the disease process, treatment options and expected results, potential adverse events or complications, and their responsibilities. The consequences of not having treatment should also be explained to the patient (3).

Systemic treatment phase

The systemic phase of periodontal treatment includes appropriate consideration of systemic diseases and their impact on the causation or treatment of disease. For example, a physician should treat individuals with systemic diseases such as blood dyscrasias before periodontal therapy is initiated, and when periodontal therapy is begun, close collaboration between the treating dentist and physician is essential. Other examples include patients who are taking calcium channel-blocking medications such as nifedipine (Procardia® or Adalat®) for cardiovascular reasons, cyclosporine (Sandimmune® or Neoral®) for auto-immune diseases or to prevent transplant rejection or phenytoin (Dilantin®) to control seizures. These medications may have the adverse effect of causing gingival enlargement (Fig. 14), and consultation with the physician regarding the possibility of using alternative medication is advisable.

Smoking has been confirmed as one of the strongest risk factors for periodontitis. Smokers should be encouraged to quit and be given the opportunity to participate in a tobacco cessation program. Diabetes (especially long-standing and poorly controlled diabetes) is associated with increased severity and extent of periodontitis. Patients with diabetes should be monitored for diabetic control and patients with severe rapidly progressive or refractory periodontitis should be referred for appropriate medical consultation for diabetes evaluation. Patients who need prophylactic antibiotics for induced bacteremia should provided with an appropriate antibiotic prescription for premedication (5, 33).

Hygienic treatment phase

This purpose of this phase of treatment is to eliminate as many of the local causes of periodontal disease as possible including bacterial plaque and calculus, faulty dental restorations and any other factors that appear to be associated with periodontal inflammation or patient discomfort. This phase includes patient education and oral hygiene instruction, extraction of hopeless teeth, placement of temporary prostheses, endodontic therapy, thorough scaling and root planing and use of local or systemic antimicrobial agents. It also includes at least temporary restoration of carious teeth and correction or replacement of defective restorations that have overhangs, open margins or open proximal contacts that result in food impaction. Final restorative care should be delayed until after all active periodontal therapy is completed because tissue contours may be altered during subsequent periodontal treatment.

It is common practice to evaluate the results of the hygienic phase of therapy approximately 6–8 weeks after its completion and make decisions for further therapy at that time. However, after scaling and root planing, periodontal healing continues for up to 4–5 months for moderately advanced periodontitis (12) and for up to 9 months for severely advanced periodontitis (13). Therefore, to take maximum advantage of the healing capacity of the peri-

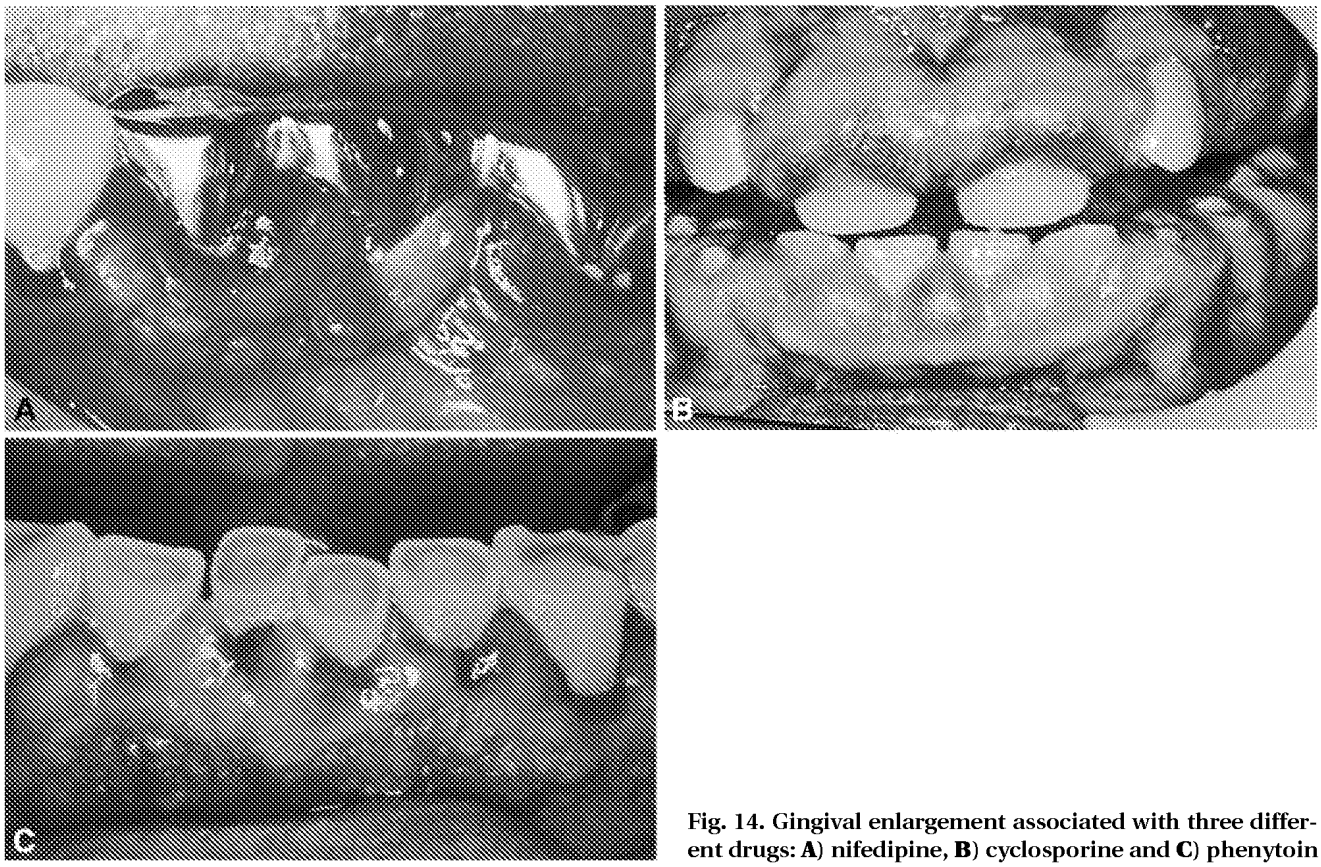

Fig. 14. Gingival enlargement associated with three different drugs: A) nifedipine, B) cyclosporine and C) phenytoin

odontal tissues, it may be best give patients supportive periodontal therapy every 3–4 months and wait 4–9 months to evaluate the results of the hygienic phase of periodontal therapy. Indeed, a number of patients who, on initial examination, appear to need periodontal surgery do not require it because of the healing that occurs as a result of hygienic therapy (76, 77). If patients do require additional treatment such as periodontal surgery, it is done in the corrective phase of therapy.

Corrective treatment phase

The corrective phase of periodontal therapy includes procedures that are designed to correct the effects of periodontal disease on the periodontal tissues, teeth and the masticatory system. This includes occlusal adjustment, fabrication of occlusal guards or biteplanes, orthodontic treatment, implant placement and periodontal surgery for debridement, resection or regeneration. As a general rule, initial healing occurs within 6 weeks after periodontal surgery, but to assure stability of tissue contours, it may be desirable to postpone final restorative care until 5–6 months after periodontal surgery (34).

Maintenance or supportive treatment phase

Supportive periodontal therapy is an essential part of any periodontal treatment plan. Periodontal therapy fails or is much less effective if it is accompanied by poor plaque control or infrequent follow-up supportive therapy (10, 18, 89, 102, 106, 117, 118). Moreover, professional tooth cleaning every 3–4 months appears to be effective for maintaining periodontal patients (27, 102). During recall appointments, the medical history is updated and the soft tissue, teeth and periodontal tissues are examined for any signs of disease and the occlusion is checked for signs of trauma. The periodontal tissues are probed, and changes in probing depth or attachment level are noted. Bleeding and suppuration on probing are also assessed, and the clinical findings are reviewed for evidence of disease progression. If there is evidence of increased loss of clinical attachment, deeper probing depths, or persistent bleeding on probing, additional therapy should be instituted. This may take the form of additional scaling and root planing, antimicrobial treatment or periodontal surgery. Moreover, patients with refractory disease may re-

quire bacteriological assessment and antibiotic therapy to control disease progression. Patients should have oral hygiene procedures reinforced and have all plaque and calculus removed from the teeth at each recall appointment. Topical fluoride treatments are administered to caries-susceptible patients, and anti-caries fluoride mouth rinses or tooth pastes should be prescribed for patients who are susceptible to dental caries.

Summary

The prevention and treatment of the periodontal diseases is based on accurate diagnosis, reduction or elimination of causative agents, risk management and correction of the harmful effects of disease. Prominent and confirmed risk factors or risk predictors for periodontitis in adults include smoking, diabetes, race, *P. gingivalis*, *P. intermedia*, low education, infrequent dental attendance and genetic influences. Several other specific periodontal bacteria, herpesviruses, increased age, male sex, depression, race, traumatic occlusion and female osteoporosis in the presence of heavy dental calculus have been shown to be associated with loss of periodontal support and can be considered to be risk indicators of periodontitis. The presence of furcation involvement, tooth mobility, and a parafunctional habit without the use of a biteguard are associated with a poorer periodontal prognosis following periodontal therapy.

An accurate diagnosis can only be made by a thorough evaluation of data that have been systematically collected by: 1) patient interview, 2) medical consultation as indicated, 3) clinical periodontal examination, 4) radiographic examination, and 5) laboratory tests as needed. Clinical signs of periodontal disease such as pocket depth, loss of clinical attachment and bone loss are cumulative measures of past disease. They do not provide the dentist with a current assessment of disease activity. In an attempt to improve the ability to predict future disease progression, several types of diagnostic tests have been studied, including host inflammatory products and mediators, enzymes, tissue breakdown products and subgingival temperature. In general, the usefulness of these tests for predicting future disease activity remains to be established in terms of sensitivity, specificity, and predictive value. Although microbiological analysis of subgingival plaque is not necessary to diagnose and treat most patients with periodontitis, it is helpful when treating patients with unusual forms of periodontal disease such as early-onset, refractory and rapidly progressive disease. There appears to be a strong genetic component in some types of periodontal disease and genetic testing for disease susceptibility has potential for future use, but more research is needed to determine its utility for use in clinical practice.

Treatment of the periodontal diseases may be divided into four phases: systemic, hygienic, corrective and maintenance or supportive periodontal therapy. Regardless of the type of treatment provided, periodontal therapy will fail or will be less effective in the absence of adequate supportive periodontal therapy.

Acknowledgments

Special thanks to Bryan Michalowicz and Daniel Pihlstrom for their suggestions and critical reading of the manuscript and to the Erwin Schaffer Chair in Periodontal Research and the Minnesota Oral Health Clinical Research Center (NICDR P-30 DE09737) for support.

References

1. Alpagot T, Wolff LF, Smith QT, Tran SD. Risk indicators for periodontal disease in a racially diverse urban population. J Clin Periodontol 1996: **23**: 982–988.
2. American Academy of Periodontology. Current procedural terminology for periodontics and insurance reporting. 7th edn. Chicago: American Academy of Periodontology, 1997.
3. American Academy of Periodontology. Parameters of care. Chicago: American Academy of Periodontology, 1996.
4. American Dental Association. Recommendations in radiographic practices: an update, 1988. Council on Dental Materials, Instruments, and Equipment. J Am Dent Assoc 1989: **118**: 115–117.
5. American Dental Association; American Academy of Orthopaedic Surgeons. Advisory statement. Antibiotic prophylaxis for dental patients with total joint replacements. J Am Dent Assoc 1997: **128**: 1004–1008.
6. Anderson KH, ed. Mosby's medical, nursing, and allied health dictionary. 5th edn. St. Louis: Mosby, 1998.
7. Armitage GC. Periodontal diseases: diagnosis. Ann Periodontol 1996: **1**: 37–215.
8. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999: **4**: 1–6.
9. Armitage GC. Diagnosis of periodontal diseases. Chicago: Committee on Research, Science, and Therapy, American Academy of Periodontology, 1995.
10. Axelsson P, Lindhe J. The significance of maintenance care in the treatment of periodontal disease. J Clin Periodontol 1981: **8**: 281–294.
11. Bader HI, Boyd RL. Long-term monitoring of adult periodontitis patients in supportive periodontal therapy: cor-

relation of gingival crevicular fluid proteases with probing attachment loss. J Clin Periodontol 1999: **26**: 99–105.
12. Badersten A, Nilvéus R, Egelberg J. Effect of nonsurgical periodontal therapy. I. Moderately advanced periodontitis. J Clin Periodontol 1981: **81**: 57–72.
13. Badersten A, Nilvéus R, Egelberg J. Effect of nonsurgical periodontal therapy. II. Severely advanced periodontitis. J Clin Periodontol 1984: **11**: 63–76.
14. Beck JD. Methods of assessing risk for periodontitis and developing multifactorial models. J Periodontol 1994: **65**: 468–478.
15. Beck JD, Cusmano L, Green-Helms W, Koch GG, Offenbacher S. A 5-year study of attachment loss in community-dwelling older adults: incidence density. J Periodontal Res 1997: **32**: 506–515.
16. Beck JD, Koch GG. Characteristics of older adults experiencing periodontal attachment loss as gingival recession or probing depth. J Periodontal Res 1994: **29**: 290–298.
17. Beck JD, Koch GG, Offenbacher S. Incidence of attachment loss over 3 years in older adults – new and progressing lesions. Community Dent Oral Epidemiol 1995: **23**: 291–296.
18. Becker W, Becker BE, Berg LE. Periodontal treatment without maintenance. A retrospective study in 44 patients. J Periodontol 1984: **55**: 505–509.
19. Belting CM, Massler M, Schour I. Prevalence and incidence of alveolar bone disease in men. J Am Dent Assoc 1953: **47**: 190–197.
20. Bergstrom J, Preber H. Tobacco use as a risk factor. J Periodontol 1994: **5**(suppl): 545–550.
21. Bolin A, Eklund G, Frithiof L, Lavstedt S. The effect of changed smoking habits on marginal alveolar bone loss. A longitudinal study. Swed Dent J 1993: **17**: 211–216.
22. Boutros SM, Michalowicz BS, Smith QT, Aeppli DM. Crevicular fluid enzymes from endosseous dental implants and natural teeth. Int J Oral Maxillofac Implants 1996: **11**: 322–330.
23. Bowers JE, Zahradnik RT. Evaluation of a chairside gingival protease test for use in periodontal diagnosis. J Clin Dent 1989: **1**: 106–109.
24. Brown LF, Beck JD, Rozier RG. Incidence of attachment loss in community-dwelling older adults. J Periodontol 1994: **65**: 316–323.
25. Burt BA, Ismail AI, Morrison EC, Beltran ED. Risk factors for tooth loss over a 28-year period. J Dent Res 1990: **69**: 1126–1130.
26. Carranza FA, Newman MG. Clinical periodontology. Philadelphia: W.B. Saunders, 1996.
27. Caton J, Proye M, Polson A. Maintenance of healed periodontal pockets after a single episode of root planing. J Periodontol 1982: **53**: 420–424.
28. Chambers DA, Imrey PB, Cohen RL, Crawford JM, Alves ME, McSwiggin TA. A longitudinal study of aspartate aminotransferase in human gingival crevicular fluid. J Periodontal Res 1991: **26**: 65–74.
29. Cianciola LJ, Park BH, Bruck E, Mosovich L, Genco RJ. Prevalence of periodontal disease in insulin-dependent diabetes mellitus (juvenile diabetes). J Am Dent Assoc 1982: **104**: 653–660.
30. Claffey N, Egelberg J. Clinical indicators of probing attachment loss following initial periodontal treatment in advanced periodontitis patients. J Clin Periodontol 1995: **22**: 690–696.
31. Contreras A, Umeda M, Chen C, Bakker I, Morrison JL, Slots J. Relationship between herpesviruses and adult periodontitis and periodontopathic bacteria. J Periodontol 1999: **70**: 478–484.
32. Corey LA, Nance WE, Hofstede P, Schenkein HA. Self-reported periodontal disease in a Virginia twin population. J Periodontol 1993: **64**: 1205–1208.
33. Dajani AS, Taubert KA, Wilson W, Bolger AF, Bayer A, Ferrieri P, Gewitz MH, Shulman ST, Nouri S, Newburger JW, Hutto C, Pallasch TJ, Gage TW, Levison ME, Peter G, Zuccaro G Jr. Prevention of bacterial endocarditis: recommendations by the American Heart Association. J Am Dent Assoc 1997: **128**: 1142–1151.
34. Dowling EA, Maze GI, Kaldahl WB. Postsurgical timing of restorative therapy: a review. J Prosthodont 1994: **3**: 172–177.
35. Ehmke B, Kress W, Karch H, Grimm T, Klaiber B, Flemmig TF. Interleukin-1 haplotype and periodontal disease progression following therapy. J Clin Periodontol 1999: **26**: 810–813.
36. Elter JR, Beck JD, Slade GD, Offenbacher S. Etiologic models for incident periodontal attachment loss in older adults. J Clin Periodontol 1999: **26**: 113–123.
37. Engebretson SP, Lamster IB, Herrera-Abreu M, Celenti RS, Timms JM, Chaudhary AG, di Giovine FS, Kornman KS. The influence of interleukin gene polymorphism on expression of interleukin-1beta and tumor necrosis factor-alpha in periodontal tissue and gingival crevicular fluid. J Periodontol 1999: **70**: 567–573.
38. Freeman JP, Brand JW. Radiation doses of commonly used dental radiographic surveys. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1994: **77**: 285–289.
39. Genco RJ. Current view of risk factors for periodontal diseases. J Periodontol 1996: **67**(10 suppl): 1041–1049.
40. Glick M, Muzyka BC, Salkin LM, Lurie D. Necrotizing ulcerative periodontitis: a marker for immune deterioration and a predictor for the diagnosis of AIDS. J Periodontol 1994: **65**: 393–397.
41. Glickman I. Clinical periodontology. 1st edn. Philadelphia: W.B. Saunders, 1953.
42. Goldman MJ, Ross IF, Goteiner D. Effect of periodontal therapy on patients maintained for 15 years or longer. A retrospective study. J Periodontol 1986: **57**: 347–353.
43. Gore EA, Sanders JJ, Pandey JP, Palesch Y, Galbraith GM. Interleukin-1beta+3953 allele 2: association with disease status in adult periodontitis. J Clin Periodontol 1998: **25**: 781–785.
44. Graves DT, Delima AJ, Assuma R, Amar S, Oates T, Cochran D. Interleukin-1 and tumor necrosis factor antagonists inhibit the progression of inflammatory cell infiltration toward alveolar bone in experimental periodontitis. J Periodontol 1998: **69**: 1419–1425.
45. Greenstein G, Lamster I. Understanding diagnostic testing for periodontal diseases. J Periodontol 1995: **66**: 659–666.
46. Grossi SG, Genco RJ, Machtei EE, Ho AW, Koch G, Dunford R, Zambon JJ, Hausmann E. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. J Periodontol 1995: **66**: 23–29.
47. Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE, Norderyd OM, Genco RJ. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. J Periodontol 1994: **65**: 260–267.

48. Haffajee AD, Socransky SS, Goodson JM. Subgingival temperature. I. Relation to baseline clinical parameters. J Clin Periodontol 1992: **19**: 401–408.
49. Haffajee AD, Socransky SS, Goodson JM. Subgingival temperature. II. Relation to future periodontal attachment loss. J Clin Periodontol 1992: **19**: 409–416.
50. Haffajee AD, Socransky SS, Lindhe J, Kent RL, Okamoto H, Yoneyama T. Clinical risk indicators for periodontal attachment loss. J Clin Periodontol 1991: **18**: 117–125.
51. Haffajee AD, Socransky SS, Smith C, Dibart S. Relation of baseline microbial parameters to future periodontal attachment loss. J Clin Periodontol 1991: **18**: 744–750.
52. Halazonetis TD, Haffajee AD, Socransky SS. Relationship of clinical parameters to attachment loss in subsets of subjects with destructive periodontal diseases. J Clin Periodontol 1989: **16**: 563–568.
53. Hancock EB, Mayo CV, Schwab RR, Wirthlin MR. Influence of interdental contacts on periodontal status. J Periodontol 1980: **51**: 445–449.
54. Hart TC, Hart PS, Bowden DW, Michalec MD, Callison SA, Walker SJ, Zhang Y, Firatli E. Mutations of the cathepsin C gene are responsible for Papillon-Lefèvre syndrome. J Med Genet 1999: **36**: 881–887.
55. Hausmann E. Digital subtraction radiography: then (1983) and now (1998). J Dent Res 1999: **78**: 7–10.
56. Hemmings KW, Griffiths GS, Bulman JS. Detection of neutral protease (Periocheck) and BANA hydrolase (Perioscan) compared with traditional clinical methods of diagnosis and monitoring of chronic inflammatory periodontal disease. J Clin Periodontol 1997: **24**: 110–114.
57. Hildebolt CF, Pilgram TK, Yokoyama-Crothers N, Fletcher G, Helbig JL, Bartlett TQ, Gravier M, Vannier MW, Shrout MK. Reliability of linear alveolar bone loss measurements of mandibular posterior teeth from digitized bitewing radiographs. J Clin Periodontol 1998: **25**(11 part 1): 850–856.
58. Hirschfeld L, Wasserman B. A long-term survey of tooth loss in 600 treated periodontal patients. J Periodontol 1978: **49**: 225–237.
59. Imrey PB, Crawford JM, Cohen RL, Alves ME, McSwiggin TA, Chambers DA. A cross-sectional analysis of aspartate aminotransferase in human gingival crevicular fluid. J Periodontal Res 1991: **26**: 75–84.
60. Ismail AI, Morrison EC, Burt BA, Caffesse RG, Kavanagh MT. Natural history of periodontal disease in adults: findings from the Tecumseh Periodontal Disease Study, 1959–87. J Dent Res 1990: **69**: 430–435.
61. Jeffcoat MK, Wang IC, Reddy MS. Radiographic diagnosis in periodontics. Periodontol 2000 1995: **7**: 54–68.
62. Jernberg GR, Bakdash MB, Keenan KM. Relationship between proximal tooth open contacts and periodontal disease. J Periodontol 1983: **54**: 529–533.
63. Jones GA, Schuman NJ, Woods MA. Estimated skin exposure as an indicator for comparing radiovisiography (RVG) versus conventional Ektaspeed Plus dental radiography. J Clin Pediatr Dent 1998: **22**: 121–123.
64. Kaldahl WB, Kalkwarf KL, Patil KD, Molvar MP. Relationship of gingival bleeding, gingival suppuration, and supragingival plaque to attachment loss. J Periodontol 1990: **61**: 347–351.
65. Kido J, Nakamura T, Kido R, Ohishi K, Yamauchi N, Kataoka M, Nagata T. Calprotectin in gingival crevicular fluid correlates with clinical and biochemical markers of periodontal disease. J Clin Periodontol 1999: **26**: 653–657.
66. Koral SM, Howell TH, Jeffcoat MK. Alveolar bone loss due to open interproximal contacts in periodontal disease. J Periodontol 1981: **52**: 447–450.
67. Kornman KS, Crane A, Wang HY, di Giovine FS, Newman MG, Pirk FW, Wilson TG Jr, Higginbottom FL, Duff GW. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997: **24**: 72–77.
68. Lamster IB. Evaluation of components of gingival crevicular fluid as diagnostic tests. Ann Periodontol 1997: **2**: 123–137.
69. Lamster IB, Grbic JT. Diagnosis of periodontal disease based on analysis of the host response. Periodontol 2000 1995: **7**: 83–99.
70. Lamster IB, ed. Diagnostic techniques in periodontology. Periodontol 2000 1995: **7**: 7–108.
71. Lang NP, Adler R, Joss A, Nyman S. Absence of bleeding on probing. An indicator of periodontal stability. J Clin Periodontol 1990: **17**: 714–721.
72. Lang NP, Joss A, Orsanic T, Gusberti FA, Siegrist BE. Bleeding on probing. A predictor for the progression of periodontal disease? J Clin Periodontol 1986: **13**: 590–596.
73. Listgarten MA, Sullivan P, George C, Nitkin L, Rosenberg ES, Chilton NW, Kramer AA. Comparative longitudinal study of 2 methods of scheduling maintenance visits: 4-year data. J Clin Periodontol 1989: **16**: 105–115.
74. Loesche WJ, Bretz WA, Kerschensteiner D, Stoll J, Socransky SS, Hujoel P, Lopatin DE. Development of a diagnostic test for anaerobic periodontal infections based on plaque hydrolysis of benzoyl-DL-arginine-naphthylamide. J Clin Microbiol 1990: **28**: 1551–1559.
75. Loesche WJ, Bretz WA, Lopatin D, Stoll J, Rau CF, Hillenburg KL, Killoy WJ, Drisko CL, Williams R, Weber HP. Multi-center clinical evaluation of a chairside method for detecting certain periodontopathic bacteria in periodontal disease. J Periodontol 1990: **61**: 189–196.
76. Loesche WJ, Giordano J, Soehren S, Hutchinson R, Rau CF, Walsh L, Schork MA. Nonsurgical treatment of patients with periodontal disease. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996: **81**: 533–543.
77. Loesche WJ, Schmidt E, Smith BA, Morrison EC, Caffesse R, Hujoel PP. Effects of metronidazole on periodontal treatment needs. J Periodontol 1991: **62**: 247–257.
78. Magnusson I, Persson RG, Page RC, DeRouen TA, Crawford JM, Cohen RL, Chambers DA, Alves ME, Clark WB. A multi-center clinical trial of a new chairside test in distinguishing between diseased and healthy periodontal sites. II. Association between site type and test outcome before and after therapy. J Periodontol 1996: **67**: 589–596.
79. Maiden MF, Tanner AC, Macuch PJ, Murray L, Kent RL Jr. Subgingival temperature and microbiota in initial periodontitis. J Clin Periodontol 1998: **25**: 786–793.
80. Mathur A, Michalowicz B, Castillo M, Aeppli D. Interleukin-1 alpha, interleukin-8 and interferon-alpha levels in gingival crevicular fluid. J Periodontal Res 1996: **31**: 489–495.
81. McGuire MK, Nunn ME. Prognosis versus actual outcome. III. The effectiveness of clinical parameters in accurately predicting tooth survival. J Periodontol 1996: **67**: 666–674.
82. McGuire MK, Nunn ME. Prognosis versus actual outcome. IV. The effectiveness of clinical parameters and IL-1 genotype in accurately predicting prognoses and tooth survival. J Periodontol 1999: **70**: 49–56.
83. Mealey B. Diabetes and periodontal diseases. J Periodontol 1999: **70**: 935–949.

84. Michalowicz BS. Genetic and heritable risk factors in periodontal disease. J Periodontol 1994: **65**(suppl): 479–488.
85. Michalowicz BS, Aeppli D, Virag JG, Klump DG, Hinrichs JE, Segal NL, Bouchard TJ Jr, Pihlstrom BL. Periodontal findings in adult twins. J Periodontol 1991: **62**: 293–299.
86. Miller PD Jr. A classification of marginal tissue recession. Int J Periodontics Restorative Dent 1985: **5**: 8–13.
87. Moss ME, Beck JD, Kaplan BH, Offenbacher S, Weintraub JA, Koch GG, Genco RJ, Machtei EE, Tedesco LA. Exploratory case-control analysis of psychosocial factors and adult periodontitis. J Periodontol 1996: **67**(10 suppl): 1060–1069.
88. Nelson RG, Shlossman M, Budding LM, Pettitt DJ, Saad MF, Genco RJ, Knowler WC. Periodontal disease and NIDDM in Pima Indians. Diabetes Care 1990: **13**: 836–840.
89. Nyman S, Lindhe J, Rosling B. Periodontal surgery in plaque-infected dentitions. J Clin Periodontol 1977: **4**: 240–249.
90. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. J Periodontol 1972: **43**: 38.
91. Okada H, Murakami S. Cytokine expression in periodontal health and disease. Crit Rev Oral Biol Med 1998: **9**: 248–266.
92. Papapanou PN. Periodontal diseases: epidemiology. Ann Periodontol 1996: **1**: 1–36.
93. Papapanou PN, Engebretson SP, Lamster IB. Current and future approaches for diagnosis of periodontal diseases. N Y State Dent J 1999: **65**: 32–37.
94. Payne JB, Reinhardt RA, Nummikoski PV, Patil KD. Longitudinal alveolar bone loss in postmenopausal osteoporotic/osteopenic women. Osteoporos Int 1999: **10**: 34–40.
95. Persson GR, Alves ME, Chambers DA, Clark WB, Cohen R, Crawford JM, DeRouen TA, Magnusson I, Schindler T, Page RC. A multicenter clinical trial of PerioGard in distinguishing between diseased and healthy periodontal sites. I. Study design, methodology and therapeutic outcome. J Clin Periodontol 1995: **22**: 794–803.
96. Pihlstrom BL. Measurement of attachment level in clinical trials: probing methods. J Periodontol 1992: **63**(12 suppl): 1072–1077.
97. Pihlstrom BL, ed. People at risk for periodontitis. J Periodontol 1994: **65**(suppl): 464–563.
98. Pihlstrom BL, Aeppli D. Problems in risk assessment in periodontal disease – use of clinical indicators. In: Bader JD, ed. Risk assessment in dentistry. Chapel Hill: University of North Carolina Dental Ecology, 1990.
99. Pihlstrom BL, Ammons WF. Treatment of gingivitis and periodontitis. Research, Science and Therapy Committee of the American Academy of Periodontology. J Periodontol 1997: **68**: 1246–1253.
100. Pihlstrom BL, Anderson KA, Aeppli D, Schaffer EM. Association between signs of trauma from occlusion and periodontitis. J Periodontol 1986: **57**: 1–6.
101. Ramfjord SP.. A rational plan for periodontal therapy. J Periodontol 1953: **24**: 75–87.
102. Ramfjord SP. Maintenance care and supportive periodontal therapy. Quintessence Int 1993: **24**: 465–471.
103. Ritchey B, Orban B. The crests of the interdental septa. J Periodontol 1953: **24**: 75–87.
104. Riviere GR, DeRouen TA, Kay SL, Avera SP, Stouffer VK, Hawkins NR. Association of oral spirochetes from sites of periodontal health with development of periodontitis. J Periodontol 1997: **68**: 1210–1214.
105. Ronderos M, Jacobs DR, Himes JH, Pihlstrom BL. Associations of periodontal disease with femoral bone mineral density and estrogen replacement therapy: cross-sectional evaluation of US adults from NHANES III. J Clin Periodontol 2000: **27**: 778–786.
106. Rosling B, Nyman S, Lindhe J, Jern B. The healing potential of the periodontal tissues following different techniques of periodontal surgery in plaque-free dentitions. A 2-year clinical study. J Clin Periodontol 1976: **3**: 233–250.
107. Safkan-Seppala B, Ainamo J. Periodontal conditions in insulin-dependent diabetes mellitus. J Clin Periodontol 1992: **19**: 24–29.
108. Schei O, Waerhaug J, Lövdal A, Arnulf A. Alveolar bone loss as related to oral hygiene and age. J Periodontol 1959: **30**: 7–16.
109. Shlossman M, Knowler WC, Pettitt DJ, Genco RJ. Type 2 diabetes mellitus and periodontal disease. J Am Dent Assoc 1990: **121**: 532–536.
110. Stoltenberg JL, Osborn JB, Pihlstrom BL, Herzberg MC, Aeppli DM, Wolff LF, Fischer GE. Association between cigarette smoking, bacterial pathogens, and periodontal status. J Periodontol 1993: **64**: 1225–1230.
111. Taylor GW, Burt BA, Becker MP, Genco RJ, Shlossman M. Glycemic control and alveolar bone loss progression in type 2 diabetes. Ann Periodontol 1998: **3**: 30–39.
112. Taylor GW, Burt BA, Becker MP, Genco RJ, Shlossman M, Knowler WC, Pettitt DJ. Non-insulin dependent diabetes mellitus and alveolar bone loss progression over 2 years. J Periodontol 1998: **69**: 76–83.
113. Tervonen T, Oliver RC. Long-term control of diabetes mellitus and periodontitis. J Clin Periodontol 1993: **20**: 431–435.
114. Theilade J. An evaluation of the reliability of radiographs in the measurement of bone loss in periodntal disease. J Periodontol 1960: **31**: 143–153.
115. Tran SD, Rudney JD. Improved multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection of *Actinobacillus actinomycetemcomitans, Bacteroides forsythus,* and *Porphyromonas gingivalis.* J Clin Microbiol 1999: **37**: 3504–3508.
116. Wang HL, Burgett FG, Shyr Y, Ramfjord S. The influence of molar furcation involvement and mobility on future clinical periodontal attachment loss. J Periodontol 1994: **65**: 25–29.
117. Westfelt E, Bragd L, Socransky SS, Haffajee AD, Nyman S, Lindhe J. Improved periodontal conditions following therapy. J Clin Periodontol 1985: **12**: 283–293.
118. Westfelt E, Nyman S, Socransky S, Lindhe J. Significance of frequency of professional tooth cleaning for healing following periodontal surgery. J Clin Periodontol 1983: **10**: 148–156.
119. Wilson R, Crouch EA. Risk assessment and comparisons: an introduction. Science 1987: **236**: 267–270.
120. Wolff LF, Pihlstrom BL, Bakdash MB, Schaffer EM, Jensen JR, Aeppli DM, Bandt CL. Salt and peroxide compared with conventional oral hygiene. II. Microbial results. J Periodontol 1987: **58**: 301–307.
121. Zambon JJ, Haraszthy VI. The laboratory diagnosis of periodontal infections. Periodontol 2000 1995: **7**: 69–82.